SOME PHYSIOLOGICAL AND ENVIRONMENTAL FACTORS LIMITING THE YIELD OF SOYBEAN

by

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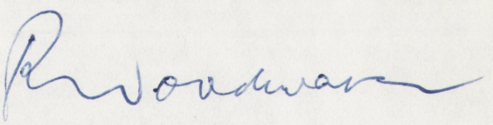
CERTIFICATE OF ORIGINALITY

The text of this thesis contains no material which has been accepted as part of the requirements for any other degree or diploma in any University or any material previously published unless due reference to this material is made. I was given much advice and aid by many people who are acknowledged elsewhere. All research was planned and performed by myself except those experiments described in Section C and Appendix II which were done in collaboration with Dr H.M. Rawson, CSIRO, Division of Plant Industry; however, all planning of these experiments, design and assembly of equipment, and analysis and interpretation of data was done jointly and equally.



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SUMMARY

A review of the literature concerning the growth and development of the soybean plant (*Glycine max* (L.) Merrill) showed that photosynthate production would probably limit the yield of beans under ideal growing conditions and that plant water deficits would probably be a common limitation to the attainment of high bean yields in the Australian environment. Some aspects of these factors were studied on soybean plants of cultivar Lee which were grown in controlled environments. Leaf photosynthesis and transpiration were determined during the expansion and senescence of leaves on plants at various stages of development growing under different light regimes, and the influence of sink size on photosynthesis and transpiration was examined. The response of the plant to a reduced water supply or an increased atmospheric demand for water was also examined.

Maximum photosynthetic rates, final leaf areas and leaf durations increased for successive leaves up to the ninth node on the mainstem but were similar for leaves from equivalent nodes on plants grown under different irradiance levels. Net photosynthesis and transpiration per unit area of soybean leaves reached a maximum at approximately the same time as the lamina reached full expansion and then declined. The fluxes of both carbon dioxide and water followed similar patterns through all stages of leaf development. Later peaks occurred in photosynthesis and transpiration which appeared to be related to flowering and pod filling and an increased requirement for assimilate at these stages. This behaviour contrasted with that of tobacco leaves where a rapid decline in photosynthetic rates before the leaf was fully expanded appeared to be related to the absence of a large demand for assimilates.

Net photosynthesis and transpiration of leaves on podded and partially depodded soybean plants were similar, and a hormone control system for photosynthesis which is partially independent of sink size is suggested. The stomatal and mesophyll (residual) resistances to gaseous diffusion behaved similarly through all stages of leaf development. Both resistances were high when the leaf was expanding or senescing and photosynthesis was low, and both were at a minimum when photosynthesis was high; possible mechanisms of this linkage are discussed. The mesophyll resistance was always the largest resistance.

A low atmospheric humidity during growth significantly reduced the bean yields of well watered plants, primarily as a result of reduced numbers of pods. Low humidity also reduced the dry weights of stems and leaves and the number of nodes on the mainstem, compared to plants grown at a high atmospheric humidity. However, the oil and protein contents of the seed were not affected by humidity levels. The results are discussed in terms of stomatal responses to humidity and leaf water deficits and reduced photosynthesis at the lower humidity. For plants grown in a glasshouse excessive water in the root zone during early pod-set encouraged vegetative growth and resulted in pod abortion and low yields. For plants which were stressed by withholding water the adaxial stomata closed earlier than the abaxial stomata in leaves going into a water deficit and the adaxial stomata opened later in leaves recovering from a water deficit. Other aspects of stomatal behaviour, such as the response to irradiance level and the effect of leaf age on stomatal resistance, were similar to what has been observed in a wide range of other species.

A consistent feature of the research was the similarity of the protein and oil contents of the seed, regardless of the treatment imposed.

Implications of this to increasing the yield of protein from the soybean is discussed. Finally, the results are discussed in the context of increasing the bean yield of the soybean plant by increasing the production of photosynthate and improving the partitioning of photosynthate to reproductive tissue. Practical limitations to these objectives are mentioned and the relevance of the results to the Australian situation is discussed.

INTRODUCTION

Shortages of food will be a problem on a world scale for many years although the specific nature of such shortages will change spatially and temporally as the weather and technology change, and not unimportantly, as man's understanding of his nutrition changes (Porter and Rolls 1973). The once widely held belief that protein shortage *per se* is responsible for undernutrition in less developed countries has been challenged and the problem is now seen to be considerably more complex, involving not only the quality of the diet but also the total energy intake of the people (Sukhatme 1973; Mauron 1973). However, there are still substantial areas of the world where the protein supply and its composition in the diet are two of the major limitations to adequate nutrition (Evans 1972).

Historically, the dietary protein requirement of Australia's population has come predominantly from animals, particularly meat (37%) and dairy products (23%) (Stillings 1973). However grazing animals use arable land inefficiently as only between 5 and 20% of plant energy is recovered as energy suitable for human consumption (Heichel and Frink 1975). The time when it will be necessary to raise this efficiency by increasing the proportion of plant energy in man's diet may not be far off. The domestic utilization of vegetable oils and protein meals has increased dramatically in recent years as a result of the increasing consumption by humans and animals (pigs and poultry) and the increasing availability of vegetable oils for industrial uses as a result of increased local crushing capacity. Since 1970 there has been a doubling

of the area sown to oilseed crops, notably *Helianthus annuus* (sunflower), *Brassica napus* (rape), and *Glycine max* (L.) Merrill -soybean.

Soybean has attributes that favour it for production in Australia compared to other new crops - a high yield of protein (≃40%) with a good balance of amino acids for human or animal nutrition, a moderately high oil content (≃20%, with 80% poly-unsaturated acids), an ability to fix nitrogen in conjunction with *Rhizobium* spp., a summer growing period making it an alternative to cotton or sugar cane, a large world market, alternative uses of the bean, a favourable price, and the benefit of a large amount of research from the United States of America on many aspects of its growth and utilization.

Soybean research in Australia commenced in the early 1890s but poor methodology and little understanding of the plant's agronomic requirements resulted in low yields and hindered progress for almost 70 years. Intensive varietal testing under irrigation since 1963 and recent agronomic studies on plant spacing and competition has nearly doubled soybean yields (from 770 kg ha^{-1} in 1967-68 to 1345 kg ha^{-1} in 1973-74) and together with high prices, has stimulated commercial interest in the crop. An estimated 53,000 hectares (70% in Queensland and 30% in New South Wales) were sown in 1974-75, an increase of 49,000 hectares since 1969-70 (Anon. 1975). Despite this increase, imports of soybean products increased to $US15.1 million in 1973-74 (Anon. 1975) with the prospect of greater usage with freer availability. However, it is not easy to obtain high yields (4-5000 kg ha^{-1}) of soybean, although farm yields averaging 3000 kg ha^{-1} are not uncommon in the U.S.A. (Shibles

et al. 1975) and have been obtained occassionally in Australia (Carter 1975). Irrigation is necessary in low effective rainfall environments, yields may be reduced by lodging, pod shattering, weed competition or insect infestation, and present commercial varieties have been bred or selected for American conditions with one exception (cv. Ruse). Some of these deficiencies could be corrected by varietal selection and breeding, providing our major limitations to production are defined and researched.

In the U.S.A., soybean is grown in areas of high rainfall and humidity where susceptibility to water deficits is not as common as in the drier Australian environment. Here the availability of an adequate supply of water is of major importance in selecting the most suitable areas for growing the crop, in specifying irrigation regimes, and in determining yields and economic returns. An estimated 71% of the Australian crop was irrigated in 1970-71. The Review of Literature will show that the drought physiology of the plant is not well understood especially in high water demand situations, and that carbon assimilation, which is dependent on an adequate plant water balance, appears to limit bean yield. One of the fundamental aims of crop research is to maximize the yield of a product of acceptable quality within the constraints imposed by limited resources. Land suitable for cropping is generally regarded as the resource which is most limiting and so increases in food production must come more from increased yields per unit area and less from expanding areas. Investigating some of the important restraints to high yields of soybean is the theme of this Thesis.

Experiments discussed in this Thesis were planned to study factors affecting photosynthesis, water balance and yield in soybean plants. The aim of the research was to study photosynthesis in relation

to plant development and the effect of some environmental and plant factors on carbon assimilation and bean yields. The aim of the work on water relations was to determine the effect of water deficits, imposed by reduced supply or increased demand at different stages of growth, on plant growth and yield.

The influence of changes in the water supply was studied in a glasshouse during summer, and differing atmospheric demand situations were studied in controlled environment cabinets; carbon assimilation was measured in both situations.

REVIEW OF LITERATURE

This review attempts to collate the knowledge on yield physiology of the soybean in order that the factors limiting yield may be identified and deficiencies defined in the context of the Australian environment. Emphasis has been placed on the interaction between carbon assimilation and bean yield and associated factors which may influence or control this relationship. I have attempted to define the major barrier to high yields in soybean, given adequate nutrition, an adapted variety and good agronomic management.

Agronomy

The soybean requires a minimum screen temperature greater than 8-10°C for growth and a maximum soil temperature (4 cm) greater than 13-15°C for germination and seedling growth (Laing and Byth 1972). The optimum temperature for leaf area and dry matter production in seedlings (cv. Biloxi) is between 27° and 33°C (Hofstra 1972). The rate of plant development prior to flowering shows a curvilinear relationship with increasing temperature with an optimum near 30°C (Brown 1960). Flower initiation and continued reproductive development in the plant is dependent upon temperature and the length of the night period (Howell 1960; Johnson *et al.* 1960), and there is considerable variation in the response of cultivars to these factors. The selection of a suitable cultivar for a particular environment is necessary for high bean yields (see Carter 1974; Laing 1974). Water deficits during flowering, pod development and pod filling may decrease bean yields (Laing 1966; Thompson 1970; Doss *et al.* 1974) so irrigation is necessary in low effective rainfall environments. A well-watered soybean crop may use between 40 and 73 cm

of water during the growing season or between 76 and 95% of pan evaporation (Peters and Johnson 1960; Dusek *et al.* 1971; Thompson 1974).

Average soybean yields have been regarded as comparatively low and attempts to increase the general yield level in the U.S.A. have not been very successful. The average annual yield increase for soybean has been 17.9 kg ha^{-1} yr^{-1} (1.3% of the mean yield, for the period 1935-68), but 102.6 kg ha^{-1} yr^{-1} (3.6% of the mean yield, for 1935-68) for maize, and 109.5 kg ha^{-1} yr^{-1} (5.7% of the mean yield, for 1944-68) for sorghum (Russell 1973). Mean soybean yield for the 1974 season in the U.S.A. was 1580 kg ha^{1}. Mean bean yield in New South Wales for 1971-72 was 1840 kg ha^{-1} (irrigated, and the highest mean yield to 1975); compared to an average wheat yield of 1340 kg ha^{-1} (dryland for 1959-69) and an average maize yield of 3220 kg ha^{-1} (1959-69). Even allowing for the higher energy content of the soybean seed, energy yields are still comparatively low (Long 1934; Hanson *et al.* 1961).

Parameters Influencing Yield

Growth of the "determinate" soybean plant can be divided into three stages. Stage I concerns vegetative growth to form the photosynthetic structure and the nutrient and water absorbing system. Maximum Relative Growth Rate (RGR) of these parts occurs during this period (Koller *et al.* 1970). Stage II involves initiation of flower primordia in leaf and branch axils, floral development and fertilization. Concurrently, the greatest (absolute) increase in leaf, stem, petiole and root dry weight per unit land area occurs (Koller 1971; Mitchell and Russell 1971). During Stage III, pod wall growth is followed by seed filling with decreasing amounts of vegetative growth (weight increase basis).

At senescence most of the leaves have abscissed leaving a bare stem with pods in racemes.

The prime determinants of yield expression are the total amount of photosynthate produced and the efficiency of utilisation of the photosynthate by the beans. The former is a function of net photosynthetic rate, leaf area, and the duration of photosynthetic activity (the assimilate source) and the latter is dependent upon the number of beans and their ability to compete for the photosynthate (the assimilate sink). The photosynthetic process is controlled by irradiance levels, while the conversion of photosynthate into plant tissue is dependent on temperature (Elmore *et al.* 1967). Other factors such as nutrient availability, the plants' response to water deficits, translocation resistance, rooting pattern, "harvest index", or canopy shape may also influence yield but are manifested through the above parameters (Curtis *et al.* 1969).

Does the sink or the source or neither limit the yield of soybean, what is the interaction between these parameters, and what environmental factors affect the limiting process?

Limitations on Bean Yield Imposed by Sink Size

Total sink size is determined by root and top vegetative growth, photorespiration, night respiration and reproductive growth. The size of the reproductive sink is determined by the number of pods per plant, the number of beans per pod and the potential size of the beans. The yield component of the sink may be too small or unable to absorb all the photosynthate produced. The following discussion considers this proposal.

Up to 81% of flowers and pods may be shed under 'normal' circumstances in the field (van Schaik and Probst 1958), but there was no evidence relating the shedding to high day and night temperatures (32°C) or long photoperiods which increased shedding in a controlled environment. Forty per cent manual depodding did not affect the bean yield of treated plants compared to control plants which aborted an apparent 10-20% of pods naturally in the field (McAlister and Krober 1958). The bean yield and pod number per plant were not affected when all floral buds were removed from one-third sections (top, middle, and bottom of the canopy) of soybean plants (Hicks and Pendleton 1969). Limited assimilate translocation to the heavily podded sections may have reduced natural shedding in the treated plants and the authors suggested that normal shedding is a result of lack of assimilates.

CO_2 enrichment (1200 µl l^{-1} of cv. Hark) during the vegetative and flowering periods increased pod numbers but not seed yield indicating that sink size was less limiting to yield than the amount of photosynthate produced during pod filling (Hardman and Brun 1971). CO_2 enrichment during pod filling increased bean yield by 25% as a result of more filled pods demonstrating the degree of sensitivity of the florets and young pods to competition for photosynthate.

Therefore, potential sink size (pod number) would not appear to limit the yield of soybeans as there are more sites for pod formation than the plant normally develops.

Source Limitations to Bean Yield

The hypothesis that the photosynthetic area (spatial and temporal), its efficiency of radiation conversion to assimilate or the

pattern of distribution of assimilate within the plant may prevent the development of all floral primordia, will now be discussed.

Photosynthetic Physiology of the Leaf Canopy. Soybean crops show a critical leaf area index (LAI) rather than an optimal LAI i.e. the lower leaves do not become parasitic on the plant and compete for photosynthate (Shibles and Weber 1965). Respiration and possibly photorespiration in these leaves are low or these lower leaves abscise (Ojima *et al.* 1965; Kumura 1969; Jeffers and Shibles 1969; Johnston *et al.* 1969). From 70 to 90% of the incident radiation on a soybean canopy is intercepted by the upper layer of leaves (Shaw and Weber 1967; Luxmoore *et al.* 1970). Increasing the irradiant flux density in the lower parts of the canopy has increased bean yield (Shaw and Weber 1967; Johnston *et al.* 1969); although the most efficient use of solar radiation by the canopy would occur when the lowest leaves are at their light compensation points. However, leaf growth in excess of that required for full radiation interception uses assimilate inefficiently and may reduce bean yield as a result of a longer vegetative period and increased water deficits (Shibles and Weber 1966) or increased lodging and plant competition (Weber *et al.* 1966).

Photosynthetic patterns measured in a field chamber led Sakamoto and Shaw (1967) to conclude the canopy was light saturated at 64.5 to 68.8 klux during initial flowering when LAI was 7, and at 59.1 klux during pod formation and filling when LAI <6. The midday plateau in photosynthesis was taken to indicate light saturation of the canopy, whereas it may have been the result of a midday water deficit or some other factor, since these results conflict with those from several other

crops. Leaves from field grown canopy plants may saturate at 107.5 klux thus the canopy would have saturated at a much greater irradiance level (Beuerlein and Pendleton 1971). At an irradiance level of 838 W m^{-2} (about 80 klux), canopy photosynthesis was light saturated at an LAI <4 and continued to increase up to an LAI >8 (Jeffers and Shibles 1969; Buttery 1970). Jeffers and Shibles (1969) suggested that the light saturation observed by Sakamoto and Shaw (1967) was a result of supra-optimal air temperatures. Egli *et al.* (1970) showed that the canopies of three cultivars of soybean were not light saturated at 838 W m^{-2} from eight weeks after sowing (the LAI was not indicated). The saturating irradiant flux density (and the maximum rate of photosynthesis) in field and cabinet grown soybeans is a function of the irradiant flux density on the leaf or plant during growth (Bowes *et al.* 1972). These authors believed acclimation to light could account for differences recorded in these parameters.

Thus the soybean canopy is characterized by a critical LAI for dry matter production, an optimal LAI for seed production under certain agronomic regimes, a poor distribution of light within the canopy, and a high irradiance requirement for light saturation, which are undesirable features for maximum productivity.

Changes in the Supply of Photosynthate and Yield. Partial defoliation of soybean canopies has generally reduced bean yields, however the amount of total leaf area removed has not decreased yield proportionately. Removal of leaves from the top, middle or bottom sections of the canopy (21%, 65% and 14% of total leaf area respectively) at the commencement of pod development decreased yields by 18%, 26% and

8% respectively (Johnston and Pendleton 1968). Row spacing was 102 cm which would over-estimate the contribution of the middle and lower leaves to yield in many commercial crops. Eighty per cent and 40% random defoliation (leaf area basis) at the commencement of pod filling reduced seed yields by 48% and 21% as a result of fewer pods per plant and lower seed weights (McAlister and Krober 1958). Light, medium and heavy defoliation at regular intervals reduced seed yields and stem and root weights in two varieties (Gibson *et al.* 1943). Partial defoliation of several species has resulted in increased photosynthesis in the remaining leaves, thus counteracting defoliation effects to some extent (Wareing *et al.* 1968 with maize and bean; Meidner 1970 with bean; Beuerlein and Pendleton 1971 with soybean; Hodgkinson 1974 with lucerne). It appears that any significant reduction in leaf area decreases bean yield in soybean as a result of a reduced supply of assimilate although mechanisms are present which may reduce such effects. Developing leaves and other vegetative parts may compete effectively with developing flowers and pods for assimilates (Weber 1968; McAlister and Krober 1968; Mann and Jaworski 1970; Hardman and Brun 1971), suggesting a limited supply within the plant.

Increasing the irradiation within a field canopy has usually increased bean yields. Johnston *et al.* (1969) increased the bean yield by 16% by placing 'Grolux' lamps within the canopy and white plastic on the soil nine weeks after sowing, with 50 cm row spacing. The response was most pronounced in the bottom and middle sections of the canopy where yield increased by 30% and 20% as a result of more beans per plant (yield did not increase in the top section). Shaw and Weber (1967) claimed that both light penetration into the canopy and yields were

increased by manual plant spreading during flowering to simulate lodging, compared to a naturally lodged canopy. Thus, increasing the irradiant flux density in the lower sections of the canopy by changing its architecture would probably increase yields through the more efficient utilization of natural light.

Yield differences between varieties are not a result of differences in photosynthetic rates but of the total photosynthate produced as determined by the duration of the pod filling period (Curtis *et al.* 1969; Dornhoff and Shibles 1970). Increased yield *within* a variety could possibly be obtained by breeding for higher rates of photosynthesis if leaf area duration was maintained.

Thus it is apparent that the bean yield of soybean plants under field conditions is limited by the amount of photosynthate available during flowering and pod set (which may result in reduced numbers of pods per plant) or during pod filling (which may result in reduced bean weights). Increases in seed yield could be obtained by increasing irradiation levels to the leaves below the peripheral layer, or through higher leaf angles (cf. Sakamoto and Shaw 1967) or changing leaf shape or plant morphology, or by increasing photosynthesis rates (Ojima and Kawashima 1968; Moss and Musgrave 1971), or by extending the pod filling period.

Arguments contrary to a source limitation can be found. Koller (1971) excluded a source limitation to yield when he concluded seed growth rate was primarily controlled by regulatory mechanisms within the seed (i.e. directly proportional to stage of seed development) rather than by the external availability of assimilates. His conclusion may be valid for seeds in a canopy where there is no source limitation

such as where the yield has been limited through flower shedding. Further data on the same crop (Koller *et al.* 1970) showed a large increase (up to 40%) in net assimilation rate (NAR) from the commencement of pod filling, indicating at least a strong interaction between seed filling and photosynthesis. Shibles *et al.* (1975) have challenged this interpretation and say the increase in NAR may have been a result of rapid leaf abscission.

Gifford (1974a) and Gifford *et al.* (1973) proposed that both the source and sink can be limiting at the same time and presented calculations of the degree of source limitation. What these authors may actually be measuring is the competitive ability of the reproductive sink compared to the vegetative sink, or a complex interaction between the two. In any case, their method is not suited to studying the soybean because of the overlap in time of the vegetative and reproductive phases of growth. Gifford's proposal that the source and the sink are both partially limiting yield is worthy of further consideration and is discussed in a later section.

Limitations to Photosynthesis

Because the supply of photosynthate limits bean yield, then with a fixed irradiance, leaf area and duration, limitations to photosynthetic rate are restricting yield. These are primarily CO_2 transfer from the atmosphere to its reduction site in the leaf, the carboxylation of CO_2 in the leaf, the rate of photorespiration, or water deficits severe enough to reduce photosynthesis.

Carbon Dioxide Transfer

The photosynthetic response of a single leaf to increasing irradiance (Fig. L1) shows a limitation by irradiance level in section A of the curve, a limitation by CO_2 transfer in section C and both factors partially limiting at B. Net photosynthetic rate per unit area (F) of individual leaves of two varieties of soybean was light saturated at 22 klux at a CO_2 concentration of 300 µl l^{-1}, but at 75 klux F increased linearly with CO_2 concentration to above 600 µl l^{-1} and the leaf was not CO_2 saturated at 1670 µl l^{-1} (Brun and Cooper 1967). The authors concluded that F was limited by the transfer of CO_2 from the atmosphere to the chloroplasts and not by cell biochemistry. Increasing the CO_2 concentration from 300 to 600 µl l^{-1} increased the mean daily photosynthesis (ground area basis) for crops of Harosoy and Wayne soybeans by 84% and 75% respectively, 60 days after sowing (Egli *et al.* 1970). Soybean plants grown in a glasshouse with air containing 350 and 1350 µl l^{-1} of CO_2 produced 50% more yield at the higher concentration owing to an increased number of pods per plant (Cooper and Brun 1967). CO_2 enrichment of field-grown Hark soybeans (1200 µl l^{-1}) for five weeks during pod filling increased seed yield by 25% (Hardman and Brun 1971). Clearly, the supply of CO_2 limits photosynthesis and yield of soybean plants. Several plant factors influence this relationship and will now be discussed.

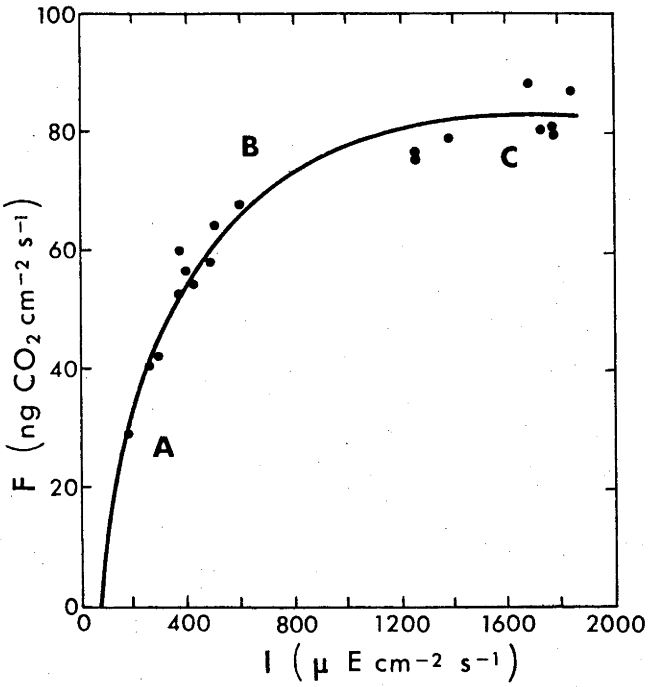

Fig. L1. Net photosynthetic rate (F) of a soybean leaflet at increasing irradiant flux density (I, 400-700 nm); data taken from Section A

Carbon dioxide transfer from the atmosphere to the reaction centres in the chloroplasts is limited by three principal resistances in series; the boundary layer resistance (r_a), the stomatal resistance (r_s), and the mesophyll resistance (r_M) consisting of physical and chemical components (Jarvis 1971).

The boundary layer resistance is dependent upon leaf size, shape, roughness and windspeed (Gaastra 1959). In assimilation chambers with air stirring r_a is generally less than 1.5 s cm^{-1} (El-Sharkawy and Hesketh 1965; Dornhoff and Shibles 1970; Upmeyer and Koller 1973). Baker and Myhre (1969, using cotton leaves) concluded r_a is comparatively low under field conditions and that (genetic) manipulation of it would be unlikely to increase F.

The stomatal and mesophyll resistances of soybean leaves are similar in magnitude under favourable conditions, although r_M is usually about twice r_s. r_s ranges from 1.5 to 15 s cm^{-1} and r_M ranges from 1.6 to 15.5 s cm^{-1} for active leaves (El-Sharkawy and Hesketh 1965; Dornhoff and Shibles 1970; Stevenson and Shaw 1971; Upmeyer and Koller 1973; Beardsell *et al.* 1973b).

Stomatal resistance appears to be important in limiting F in soybean. Reduced transpiration as a result of stomatal closure has been accompanied by parallel decreases in F (Boyer 1970b; Beardsell *et al.* 1973a).

Gaastra (1959) believed that r_M was an important yield-determining factor in crop plants because of its relatively high value, and in soybean Dornhoff and Shibles (1970) and Beardsell *et al.* (1973b) have shown r_M to be the most limiting resistance to F. The physical transfer component of r_M is the major limiting resistance in some circumstances (Gaastra 1959; Brun and Cooper 1967; Kriedemann *et al.* 1970; Jones and Slatyer 1972). However in two similar experiments on cotton, Jones and Slatyer (1972) found the transfer component was the major resistance in one and the carboxylation component in the other and suggested that both components are linked.

In soybean (and other C_3 plants) CO_2 reacts with ribulose-1-5-diphosphate (RuDP) to form 3-phosphoglycerate in the presence of RuDP carboxylase, in the chloroplast. It has been suggested that this or associated reactions may also limit or control F. The evidence is somewhat indirect. Hesketh (1963) originally proposed that the different productivity between several species was a result of differences in r_M or in the kinetics of the dark reactions. Partial defoliation of birch

increased F of the remaining leaves within a few days (Sweet and Wareing 1966). This was later demonstrated on other species on fully expanded leaves (Woolhouse 1968; Hodgkinson 1974) and the increase in F was associated with increased activity of RuDP carboxylase and increased supply of cytokinins from the roots (Wareing *et al.* 1968). Part of the increase in F was probably attributable to increased demand for assimilate on the remaining leaves, and some of the increase (of the order of 30%) could also have been attributable to increased stomatal conductance (Meidner 1970). However, the lower r_s may be directly attributable to the lower r_M and the subsequent lower substomatal concentrations of CO_2 (Meidner and Mansfield 1965). Meidner (1970) also showed that debudding *Xanthium* increased F in the leaves as a result of changes in r_M, not r_s. A close relationship was found between F and RuDP carboxylase activity in the first eight leaves of *Capsicum* (Steer 1971), in leaves of cabinet grown soybean (Bowes *et al.* 1972), and in bean (Wareing *et al.* 1968), and cf. Bjorkman (1968).

Therefore F is also limited by the activity of carboxylation enzymes in the chloroplasts. It is possible the transfer and carboxylation components of r_M both partially limit F.

It is also clear that both r_s and r_M partially limit F. Unless CO_2 concentration in the canopy can be increased, which is not feasible with present technology (Allen *et al.* 1974), finding varieties with more efficient F at 300 $\mu l\ l^{-1}$ CO_2 via lower stomatal and mesophyll resistances would be worthwhile.

Photorespiration

Photorespiration is a light induced CO_2 release process involving glycolate metabolism and requiring oxygen (Jackson and Volk 1970) and is difficult to estimate because of the inadequacy of present methods (Ludlow and Jarvis 1971). The rate of photorespiration of soybean leaflets has been estimated at between 17 and 45 ng CO_2 cm^{-2} s^{-1} (Forrester *et al.* 1966; Samish *et al.* 1972) depending on the method used. This represents between 7% and 30% of F.

Clearly, reducing photorespiration would increase net photosynthesis in leaves, but attempts to find soybean varieties without photorespiration have been unsuccessful (Cannell *et al.* 1969). In fact, Hofstra and Hesketh (1969) and Jackson and Volk (1970) have suggested that photosynthesis and photorespiration are closely associated which may mean that one can not only be easily reduced without affecting the other, although Samish *et al.* (1972) found no such relationship over several varieties of soybean.

Water Deficits and Photosynthesis

Water serves at least four functions in plant growth. It is a major constituent of living tissue, a reagent in photosynthesis, a solvent for salts, sugars and gases, and aids the maintenance of cell turgidity (Kramer 1963). Transpired water also helps to prevent high temperatures in some tissues which could affect enzyme action. Reduced water availability can have important consequences on photosynthesis and leaf growth (Boyer 1970a) and thus the total production of photosynthate.

Water deficits may reduce F through stomatal closure and the subsequent increase in CO_2 diffusion resistance and through cell biochem-

istry. The broad, planar leaves of soybean intercept large quantities of irradiation which promotes leaf water deficits (Stevenson and Shaw 1971). Ghorashy *et al.* (1971) claimed that photosynthesis in the soybean plant is more sensitive to stress during pod filling than during flowering. This may be true in absolute units of F but the three isolines which were studied exhibited between 74 and 120% higher F during pod filling than during flowering. The relative decrease in F in all three isolines at both stages of growth was similar (≈57%) with only one exception, as a result of a drop in leaf water potential (LWP) from -8 or -12 bars to -20 bars. F decreased linearly with decreasing LWP. F decreased when relative turgidity (RT) fell below 90%, and was half the maximum rate at 79-84% RT for soybean leaves (Chen *et al.* 1971; Laing 1966) - Fig. L2. F of leaves of cv. Harosoy was unaffected by desiccation until LWP dropped below -11 bars (Boyer 1970a,b). Large increases in r_s have been observed when LWP dropped below -13 bars (Teare and Kanemasu 1972). F was controlled solely by stomatal behaviour down to a LWP of -16 bars where F was 60% that of a well-watered plant (Boyer 1970b). r_M remained low, ≈6 s cm^{-1} (24% of the total resistance, $r_a + r_s + r_M$) at desiccation levels of -41 bars. r_M in cotton was similarly unaffected when RT dropped from 92 to 56% (Troughton and Slatyer 1969). Biochemical pathways were not affected by water stress down to -12 bars in *Pisum* (Boyer and Bowen 1970) and again stomatal aperture had the greatest influence on F.

A major criticism of such work is that the plant is usually well watered until the stress is applied, and the stress is applied quickly, a situation unlikely to be found in the field. Field grown sunflower and sorghum plants adapt to water deficits, so that the LWP at

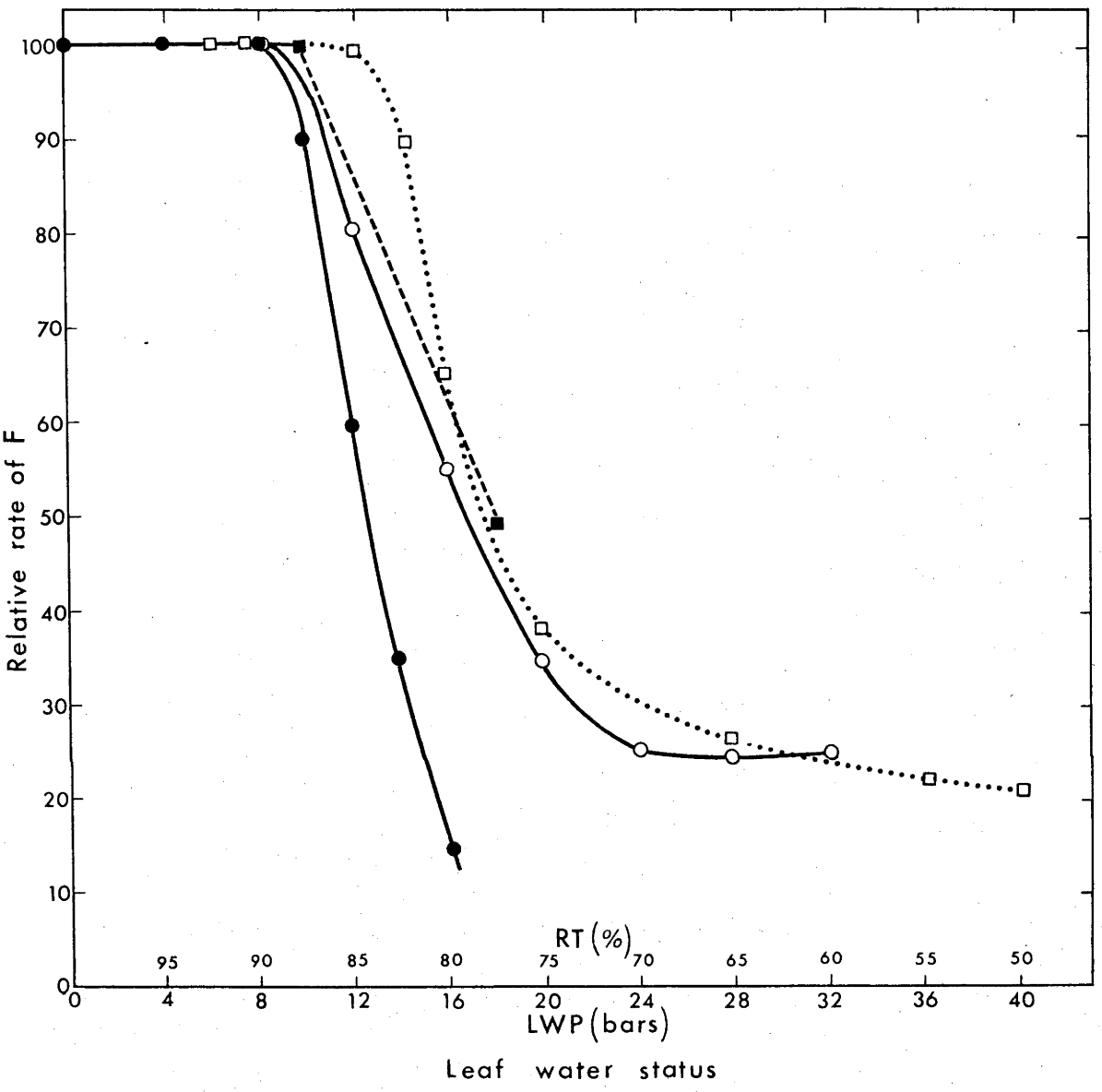

Fig. L2. The relationship between relative net photosynthetic rate (F) and water status of soybean leaves from Laing (1966, ●), Chen *et al.* (1971, ○), Boyer (1970, □), and Ghorashy *et al.* (1971, ■). The first two references use relative turgidity (RT) and the last two leaf water potential (LWP)

which the stomata close gradually decreases e.g. in water stressed sunflower grown in the field the stomata closed at a LWP between -21 and -27 bars (Turner pers. comm.) compared to -12 bars suggested by Boyer (1970a).

However, it is clear that water deficits increase primarily r_s and reduce leaf expansion, with subsequent reductions in photosynthesis and probably yield.

These aspects of soybean physiology which appear to limit or reduce photosynthesis and yield will be examined in the remaining sections of the Thesis. More specific Literature Reviews are presented in the appropriate sections.

Turner, N.C., Experiments on adaptation of sunflower and sorghum to water deficits. (1975).

SECTION A: THE EFFECT OF WATER DEFICITS ON PHOTOSYNTHESIS, STOMATAL BEHAVIOUR AND YIELD OF SOYBEAN

Abstract

Soybean plants of cv. Lee were grown in soil in large bins in a glasshouse and a water deficit was imposed by withholding water at the pre-flowering, flowering, or pod filling stages of growth. Stomatal diffusive resistances and net photosynthetic rates were measured on individual leaflets of stressed and well watered plants. Dry matter accumulation and bean yields were determined.

The responses of the adaxial and abaxial stomata to irradiance levels and plant water deficits were similar to those of other species. The adaxial stomata were more sensitive to environmentally imposed stresses than the abaxial stomata. Increased stomatal resistance in older leaves was associated with a decline in rates of photosynthesis. Leaf photosynthesis was variable and this may have been an effect of leaf age or leaf position on the plant.

Bean yields were not significantly affected by any of the stress treatments compared to the yields of well-watered plants. Excessive water in the root zone at early pod-set and high relative humidities may have limited the bean yield in all treatments by encouraging vegetative growth at the expense of reproductive growth. Possible reasons for this are presented. The methodology of the experiment and some aspects requiring further examination are discussed.

INTRODUCTION

This first experiment was to provide information on specific aspects of soybean physiology on which to base later work. It was used to develop techniques for growing soybean in controlled environments and for obtaining data on the relationship between leaf photosynthesis and yield under a range of droughting regimes.

Water stress is a common and important factor influencing soybean production in the Australian environment, even when the crop is grown with irrigation (Carter 1975). The resistance to water transport in whole soybean plants is twice that of sunflower or bean, as a result of a high radial resistance in the root (Boyer 1971). Thus leaf water potential has to drop twice as low in soybean to maintain a given rate of water transport to the leaf. (Barrs (1973) tentatively suggested that the root resistance in cotton and tomato may decrease under high transpiration rates, thus helping to prevent the onset of stress associated with plant resistance, although Boyer (1974) claimed that the change in resistance is predominantly a leaf phenomenon in sunflower.) Thus, a high atmospheric demand and/or low soil moisture availability will result in soybean crops experiencing water deficits more rapidly and more often than other crops.

A short-term water deficit during pod filling reduces yields by a greater amount than water deficits at other growth stages of soybean, as a result of reduced pod numbers and reduced bean weight (Laing 1966; Dusek *et al.* 1971; Doss *et al.* 1974 and cf. Thompson 1970). This period is more critical than others because no compensation for poor bean filling can occur since the plant is approaching maturity. Water deficits during flowering may reduce yield as a result of fewer

pods per plant, but compensation may occur in other yield parameters (e.g. more beans develop per pod or bean weight is increased). The lower yields are generally associated with reduced assimilate production, as a result of high stomatal resistances and low leaf water potentials (Denmead and Shaw 1960; Laing 1966; Boyer 1970b). The absolute reduction in photosynthetic rate as a result of a water deficit can be greater during pod filling than during flowering (Ghorashy *et al.* 1971), although the relationship between reduced rates of photosynthesis and reduced bean yield requires elucidating.

The aim of the experiment was to determine the effect of a water deficit applied during the pre-flowering, flowering or pod filling stages of growth on stomatal behaviour, photosynthesis and yield, and to determine the extent to which these factors are interrelated.

MATERIALS AND METHODS

Plant Culture

Graded (220-230 mg), pre-germinated seed of soybean cv. Lee was sown without *Rhizobium* inoculant into a fertile sandy-loam soil in 0.05 m^3 bins in a glasshouse at Canberra. Seedlings emerged on November 17, 1972 and twelve days later (the second trifoliolate leaf was unfolding) were thinned to one plant per bin and were graded by size into three blocks, each of eight bins spaced 1 m apart. Watering was initially from the top but as transpiration rates increased a constant head watering system was established on January 22, 1973. This maintained a water table in a layer of coarse river sand in the bottom of each bin. The plants received nutrient solution (modified Hoaglands) weekly. Glasshouse day/night temperatures were 30/25°C ($\pm$1°C) for 12/12 h under a natural photoperiod.

Application of Water Deficits and Plant Harvests

One plant in each block was water stressed at one of three stages of growth - pre-flowering, flowering, or pod filling - by withholding water from the bin until leaves wilted. The rest of the plants were always well watered.

Pre-flowering Stress

Water was withheld from three plants from December 15 (28 days after emergence, when the plants had seven nodes on the mainstem) and wilting was first evident on December 20. On December 23 there was little overnight recovery of leaf turgor or stomatal resistances so the stressed plants were rewatered. One non-stressed plant from each block was selected at random and harvested for dry weights of stems and leaves (after drying at 85°C for 24 h) and total leaf area (measured with an electronic planimeter).

Flowering Stress

Plants commenced to flower about December 30. Water was withheld from three plants from January 8, 1973 (52 days from emergence) when flowers were present on all nodes except the second from the top. Leaves were wilting on the afternoon of the following day and as recovery overnight was partial and temporary the plants were rewatered at 1800 h on January 10. One non-stressed plant was harvested from each block on January 11. The same parameters were measured as previously and the number of inflorescences was counted. Pod formation commenced about January 19 (day 63) and pod filling about February 8 (day 83).

Pod-filling Stress

The pod-filling stress was imposed by slowly lowering the water table in the bottom of the bins from February 23 (98 days after emergence). This was to prevent the stress developing too rapidly because of the large leaf area. The plants started to wilt on March 5 and since the symptoms were evident the following morning, the plants were rewatered at 1200 h. Two well-watered plants were harvested (the third had previously been rejected because of a bacterial stem infection) and the dry weights of plant parts and leaf areas were determined.

Final Yield Harvest

Pods had matured by May 2 (166 days from emergence) but the leaves remained light green and had not senesced by June 4 when watering ceased. Three well-watered (control) plants and all stressed plants were harvested on June 20 and the dry weights of plant parts and the yield components were determined.

Measurement of Stomatal Resistance

Stomatal resistances to water vapour diffusion (r_s) were measured with an aspirated diffusion porometer (Byrne *et al.* 1970). Both surfaces of two recently expanded terminal leaflets were measured on each control plant and each stressed plant usually on three occasions each day during the periods when the water deficits were applied. The irradiant flux density was measured on the adaxial surface in the plane of the leaf with a filtered silicon photocell (McPherson 1969) calibrated against a Lambda Instruments quantum sensor.

Measurement of Net Photosynthesis

Net photosynthetic rates of recently expanded terminal leaflets were measured during flowering and pod filling in the glasshouse using infra-red gas analysis in an open system with a single-leaf assimilation chamber. The apparatus is described in detail in Appendix 1. A north-facing leaflet was chosen each day for measurement. Mean leaflet temperature at daily maximum photosynthetic rates was 30.1 ± 0.2°C and mean vapour pressure deficit was 16 mb. Measurements usually commenced about 0900 h and ended before 1600 h, but as the season progressed this period decreased because of the altitude of the sun.

Daily total short-wave irradiation (400-1100 nm) in the glasshouse was measured with an integrating silicon cell pyranometer (accuracy ± 10%) - Fig. A1.

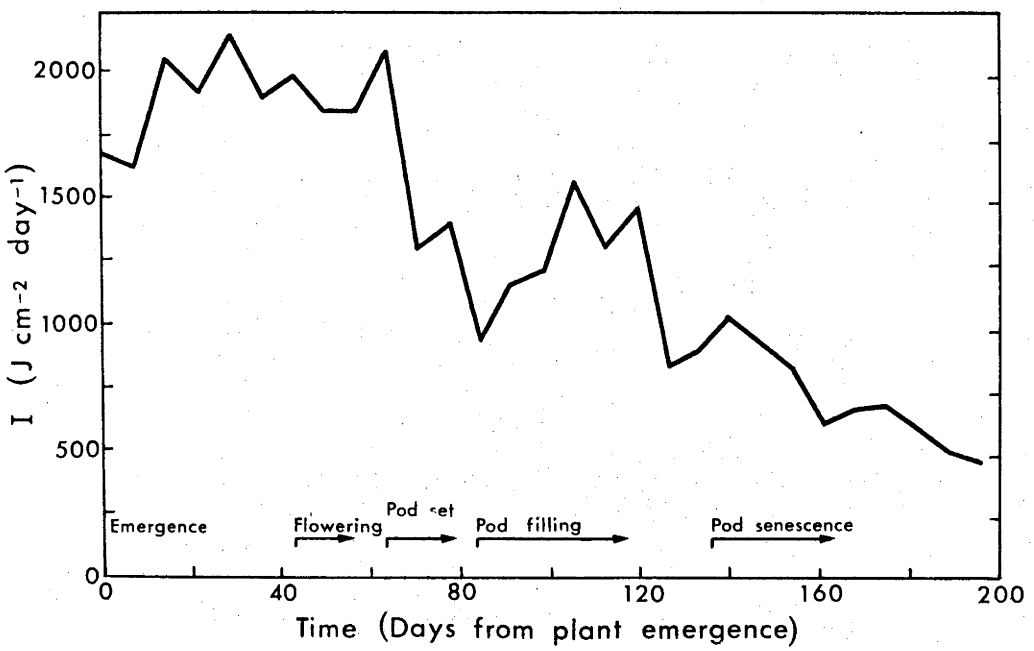

Fig. A1. Mean total daily shortwave (400-1100 nm) irradiance (I) in the glasshouse for seven day periods and plant phenology from emergence

RESULTS

Stomatal Response to Irradiance Level

The irradiance level as well as the water status of the leaf may influence r_s, therefore it is necessary to first examine the response of the stomata to changes in irradiance in order to separate the influence of these factors. The stomatal resistances for both surfaces of leaves from well watered plants at three growth stages is shown in Figs A2a and A2b. The regressions were fitted to the data using the model

$$Ir_s = Ir_{min} + I_m r_{min}$$

where r_{min} is the minimum resistance and I_m is the irradiance at $2r_{min}$ (and see Turner and Begg (1973) for use of this model with tobacco and grass leaves). The irradiance levels were measured on the adaxial leaf surface so the abaxial stomata would actually have been under a lower irradiance than that quoted. Thus, the abaxial stomata opened at much lower irradiance levels than the adaxial stomata at all stages of growth. The resistance of the adaxial stomata was generally from two to three times greater and was more variable than that of abaxial stomata during the pre-flowering and flowering stages at all irradiance levels. Stomatal resistances were similar during the pre-flowering and flowering stages but were considerably higher during the pod-filling stage. During pod filling the adaxial stomata remained closed, in well watered plants, independent of the irradiance level.

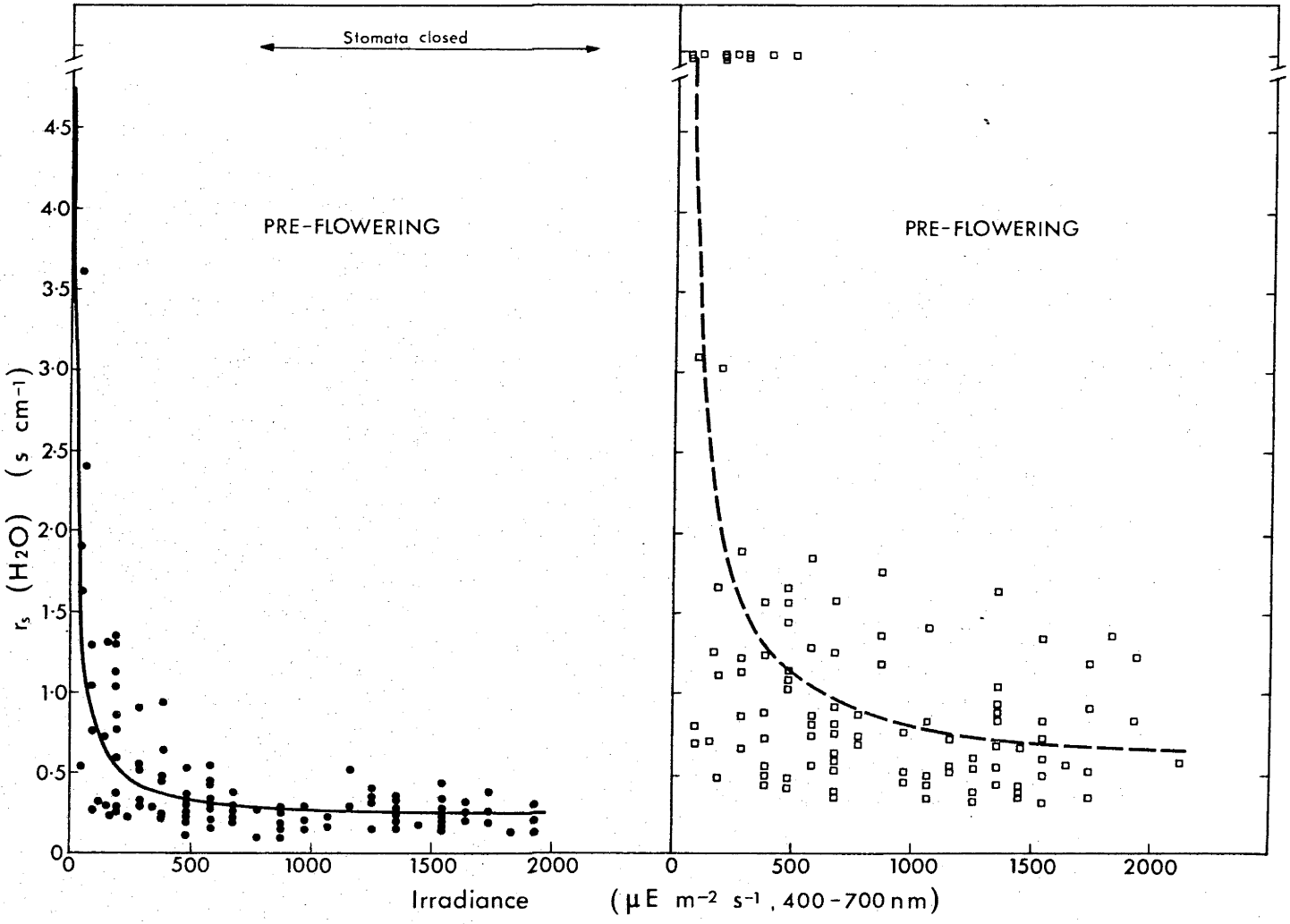

Fig. A2a. Stomatal resistance to water vapour diffusion (r_s) for the adaxial (□) and abaxial (●) leaf surfaces plotted against irradiance level for adequately watered soybean plants during the pre-flowering stage of growth; lines were fitted by regression

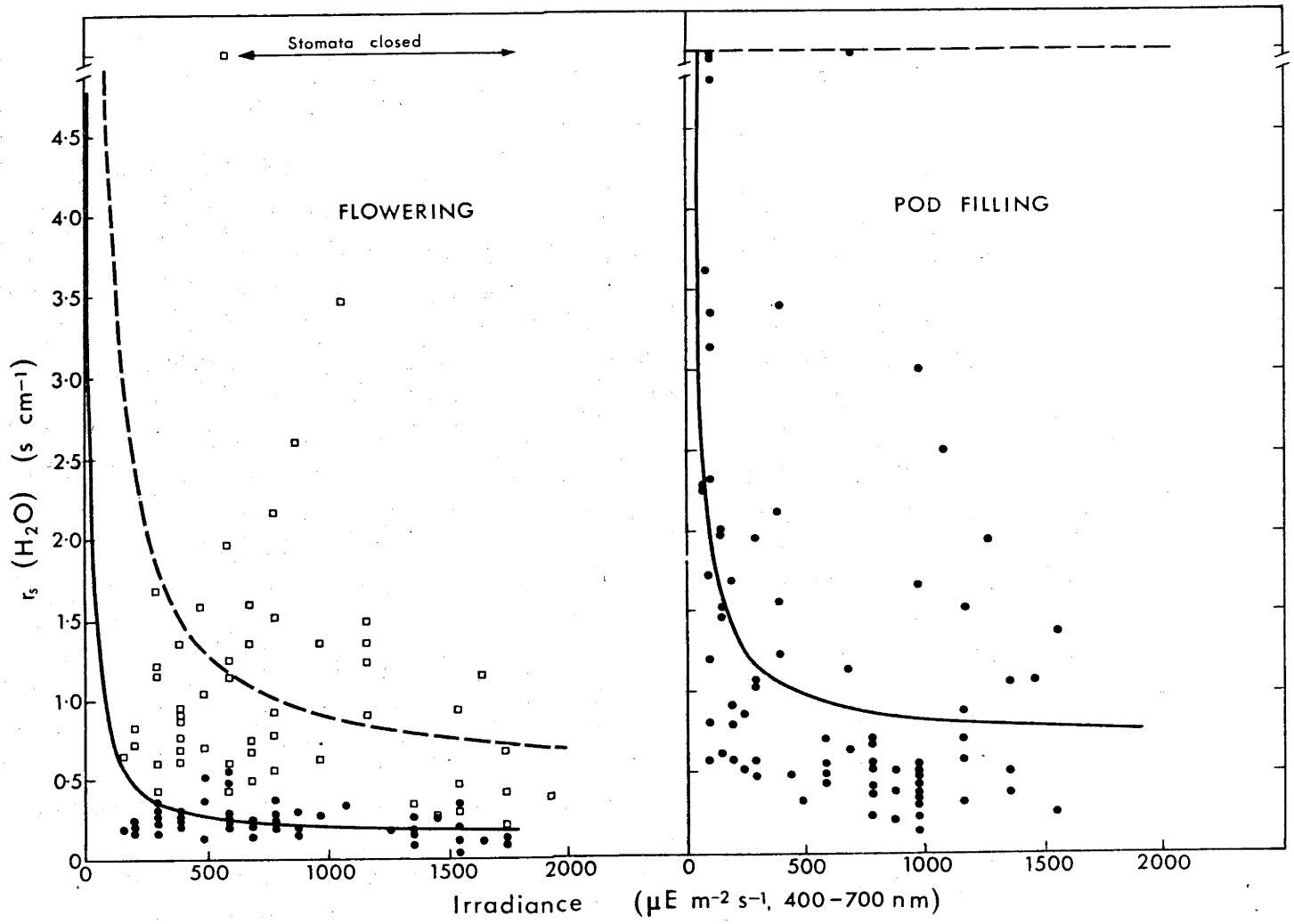

Fig. A2b. Stomatal resistance to water vapour diffusion (r_s) for the adaxial (□) and abaxial (●) leaf surfaces plotted against irradiance level for adequately watered soybean plants during the flowering and pod filling stages of growth; lines were fitted by regression

Points of Clarification

Two points of clarification are required. First, the porometer measured the combined effect of the stomatal and cuticular resistances to water vapour diffusion. Leaves with completely closed stomata produced no readings on the porometer (even after hundreds of seconds) and this is taken to indicate the cuticular resistance of these soybean leaves was large enough to be neglected and thus the porometer was effectively measuring r_s.

Second, the r_s values are low when compared to those from an assimilation chamber (Section C) from or other workers e.g. Dornhoff and Shibles (1970), Stevenson and Shaw (1971) and Teare and Kanemasu (1972). However, calibration of the porometer was by the method recommended by Byrne *et al.* (1970) and was checked several times and agreed with their calculations. The diffusive resistance of slit-shaped stomata can be calculated using theory proposed by Jarvis *et al.* (1967, equation 11a) and from data on stomatal frequency and dimensions obtained on December 23 (stomatal frequency 28100 cm^{-2}, depth 12 x 10^{-4}cm, length 11 x 10^{-4}cm, width 5 x 10^{-4}cm for the abaxial stomata; and frequency 11700 cm^{-2} and length 12 x 10^{-4}cm for the adaxial stomata). The calculated r_s values (to H_2O at 30°C) were 0.28 and 0.63 s cm^{-1} for abaxial and adaxial stomata and are similar to those measured with the porometer. There would appear to be some factor associated with the porometer which under-estimates r_s relative to an assimilation chamber. Positioning the porometer away from leaf margins or large veins and midway down the leaf may be part of the cause. However, since all stomatal measurements derived with the porometer for this Thesis (Sections A, B and D) are used for comparative purposes only, the interpretation of the results is not affected.

Stomatal Response to Water Deficits

The resistance of the adaxial and abaxial stomata for leaves on well-watered and stressed plants during the three periods of water deficit is shown in Fig. A3. The measurements were made between 0900 and 1000 h except those during pod filling which were taken at 1100 h. Each point is a mean of five or six measurements on three plants at irradiance levels greater than 500 $\mu E\ m^{-2}\ s^{-1}$ (except where indicated on the figure during pod filling). Standard errors were very small.

The pre-flowering stress developed slowly because of the small leaf area of the plants. The resistance of the adaxial stomata increased 48 h before that of the abaxial stomata. The plants were re-watered after all stomata had closed and leaves had lost turgidity, no recovery data are available.

During the flowering stress the resistance of the adaxial stomata again increased before that of the abaxial stomata but this is obscured because only the 0900 h readings are presented. This stress developed more rapidly than the previous one because the larger leaf areas transpired the limited amount of water at a faster rate. The abaxial stomata were first to respond to re-watering whereas the adaxial stomata had not fully recovered four days later despite the leaves appearing turgid 16 h after re-watering.

The adaxial stomata on stressed and well-watered plants remained closed or nearly so during pod filling. The resistance of the abaxial stomata increased as the stress developed; on re-watering the plants the resistance did not start to decline for about seven days and did not reach the lower values of well-watered plants. This appeared to be a result of a disruption of stomatal function as measurements taken

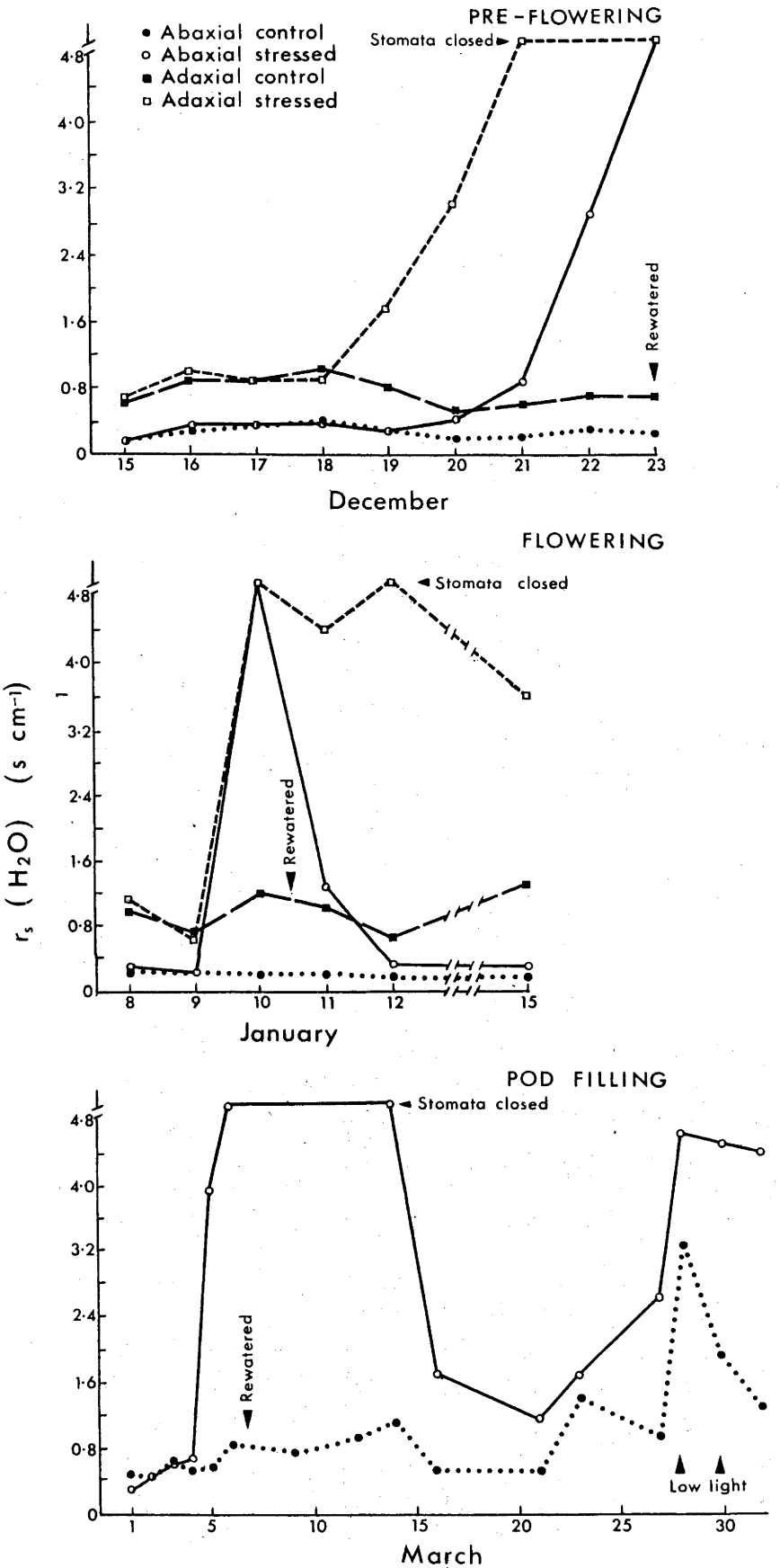

Fig. A3. Stomatal resistance to water vapour diffusion (r_s) for abaxial and adaxial surfaces of stressed (open symbols) and well hydrated (closed symbols) soybean leaves at 0900 h or 1100 h during the periods of water deficits. Mean of three plants; irradiance level >500 $\mu E\ m^{-2}\ s^{-1}$

between March 28 and April 3 with a pressure chamber showed no significant difference in the water potentials of leaves from well-watered and stressed plants (7.9 $\pm$ 1.4 bars and 12.1 $\pm$ 2.9 bars respectively).

Net Photosynthesis during Flowering and Pod Filling

The maximum net photosynthetic rate per unit leaf area (F) recorded on each day of measurement on well-watered and stressed plants is shown in Fig. A4. Photosynthetic rates were maintained until during pod filling and then declined, although the data are variable. Since the leaves remained green for about two months after measurements ceased, F probably remained low during this period (cf. the similar behaviour of leaves on partially depodded plants, Section C).

The photosynthetic rates of leaves on plants in the well-watered and stress treatments were comparable prior to the application of stress. Stress developed to the point where there was no net flux of CO_2 from the leaf which indicates complete stomatal closure (supported by the measurements of r_s).

Leaf resistance (r_l) to water vapour diffusion was calculated from $1/r_l = 1/r_d + 1/r_b$, where r_d and r_b are the stomatal resistances of the adaxial and abaxial leaf surfaces. The decline in leaf photosynthesis of control plants from about day 100 was associated with an increase in r_l - Fig. A4. Each r_l value is a mean of measurements on two leaves on each of three plants on three occasions during the day (for irradiance levels greater than 500 $\mu E\ m^{-2}\ s^{-1}$).

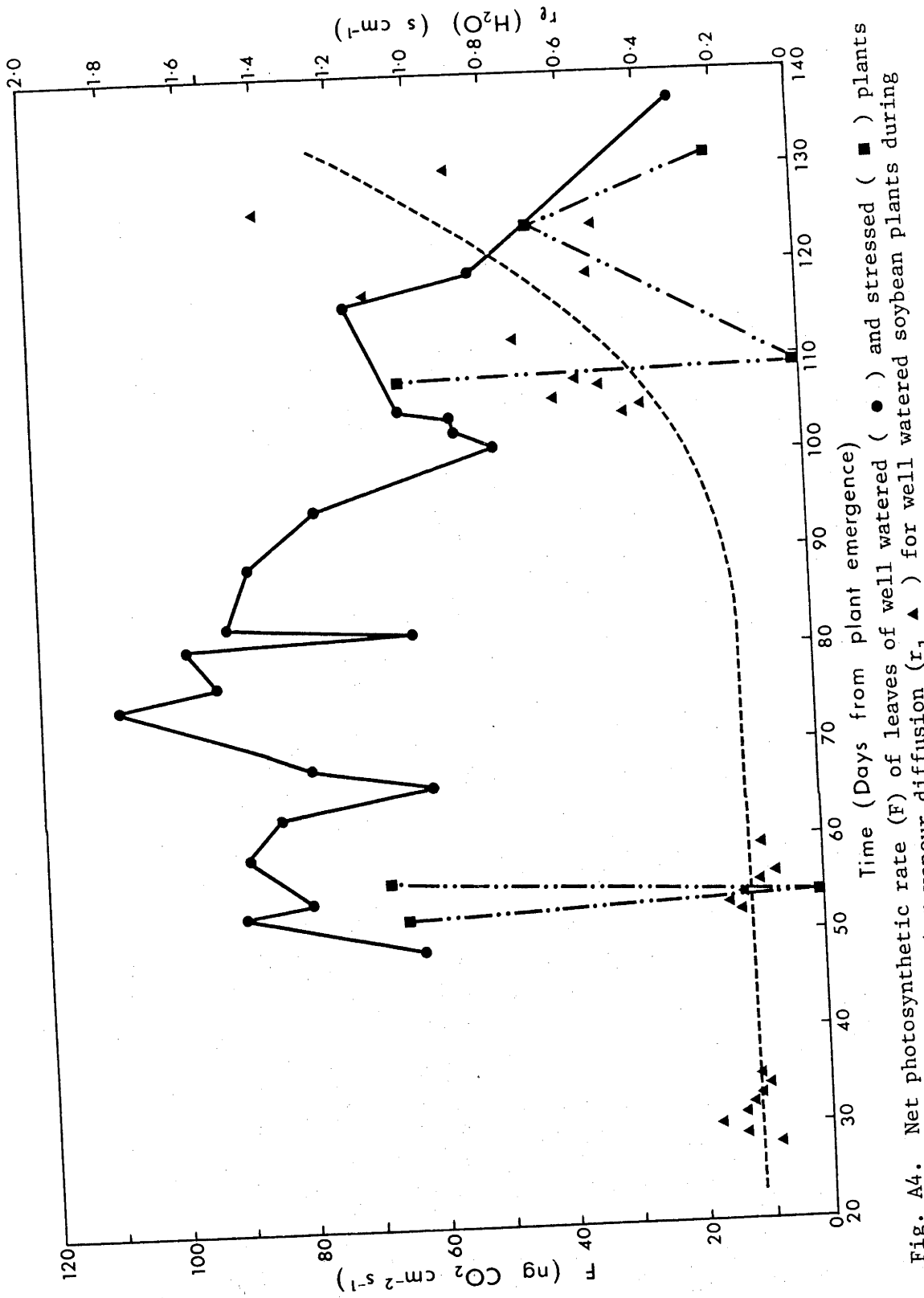

Fig. A4. Net photosynthetic rate (F) of leaves of well watered (●) and stressed (■) plants and leaf resistance to water vapour diffusion (r_l ▲) for well watered soybean plants during flowering and pod filling

Bean Yield and its Components

The bean yields of the plants were low and there was no significant effect of treatment on bean yields (at the 0.05 level of probability) - Table A1. The 'harvest index' of the control plants was less than 6%. The plants which were stressed during flowering produced the highest bean yield while those stressed during pod filling yielded the least. This latter stress completely stopped pod filling (cf. Fig. A5, day 109) and caused the death of about 70% of the leaves.

The only yield component significantly affected by the treatments was the 100 bean weight. Bean weight was highly correlated to bean yield ($r^2 = 0.96^{**}$). The number of pods per plant from the four treatments was similar at maturity (although many pods could have been shed before the harvest) and only about half the pods on all plants contained beans.

Bean Quality

The mean oil and protein content of the beans over all treatments were 21.1% and 46.6% respectively (Table A1). Neither parameter differed significantly between treatments. The high oil content may be associated with the relatively high temperatures in the glasshouse (Howell 1960).

Dry Matter Accumulation

The well-watered plants produced high leaf areas and branched heavily (Fig. A5). The most notable feature of this data is the 34% increase in the dry weight of tops from three weeks after pod filling commenced (day 109) until senescence; 83% of which was stem and leaf growth and the remainder was pod and bean growth. The area and number

Table A1 Bean yield, bean yield components and bean quality parameters (and least significant differences where the treatment effect was significant) for soybean plants which were water stressed at three stages of growth

	Stress period				LSD
	Control	Pre-flowering	Flowering	Pod filling	P<0.05 P<0.01
Bean yield (g)	75.8	85.0	153.7	25.1	
Pod no. per plant (with beans)	359	308	441	224	
Bean no. per pod	2.10	2.15	2.07	1.58	
100 bean weight (g)	10.07	12.84	16.88	7.11	3.9 6.0
Bean no. per plant	753	662	911	353	
Pod no. per plant[A] (incl. aborted)	734	688	721	639	
Protein content (%)	46.6	47.5	46.6	45.6	
Oil content (%)	20.9	21.2	21.9	20.5	

[A] This is the total number of pods on the plant at the final harvest; it does not include those that abscissed.

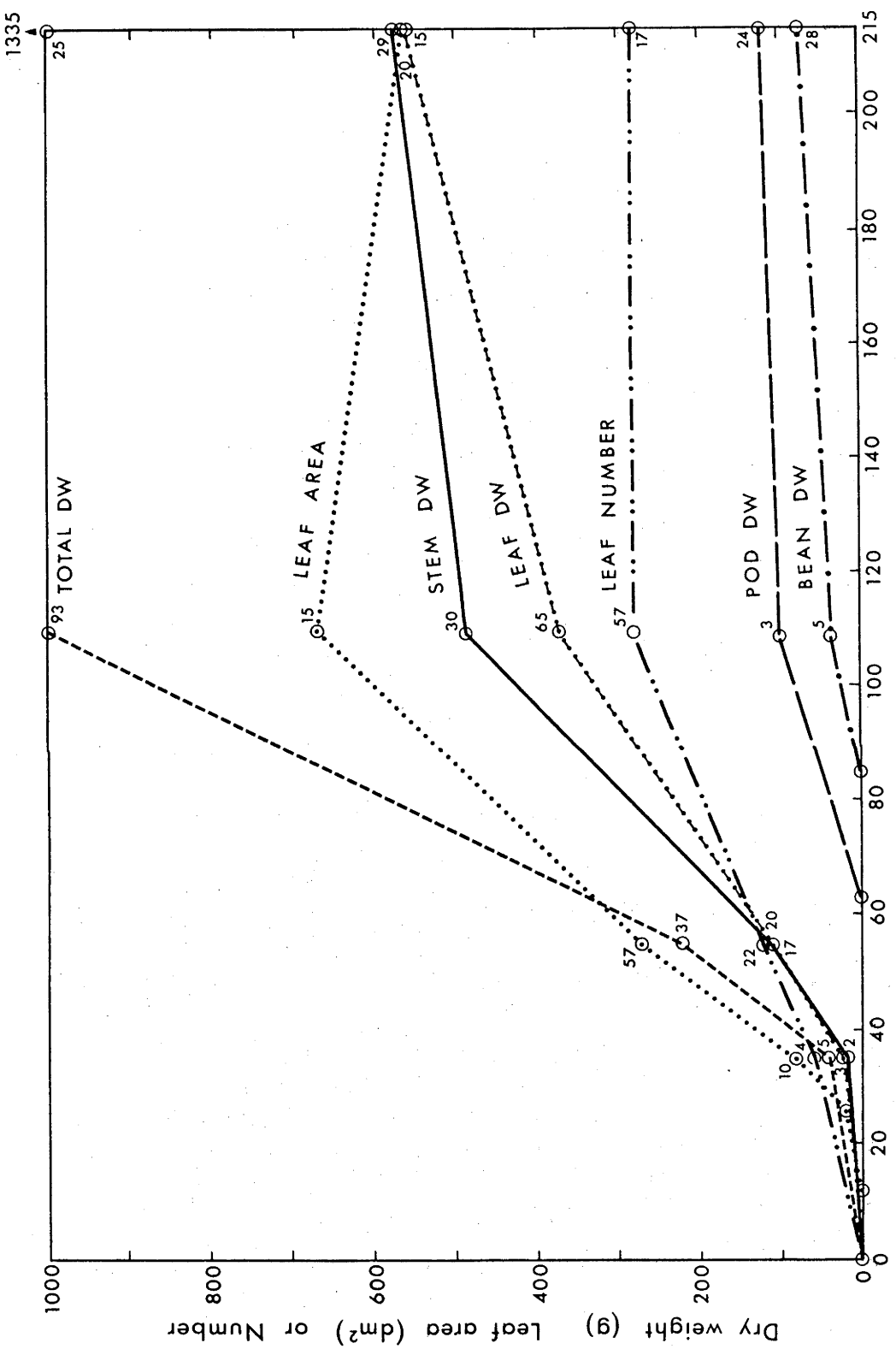

Fig. A5. Dry weight (DW) accumulation of plant parts, leaf areas and numbers of leaves for well watered soybean plants from emergence (day 0) to maturity; standard errors for each mean are indicated

of leaves did not change during this period but the specific leaf weight did - from 4.43 mg cm^{-2} on day 55, to 5.57 mg cm^{-2} on day 109 and 9.90 mg cm^{-2} on day 215. The increase in dry weight of vegetative parts during pod filling may also have occurred in the plants stressed at pre-flowering and flowering because their bean yields were also low.

DISCUSSION

Stomatal Behaviour in Well-watered and Stressed Plants

Irradiance level, leaf water balance, atmospheric humidity and carbon dioxide concentration may influence stomatal resistance, but only irradiance and water supply to the plant are considered here since changes in resistance as a result of variation in the other factors may be relatively small in the glasshouse (Brun and Cooper 1967; Schulze *et al.* 1972; Turner 1974).

The stomata responded similarly to those of other species as diverse as tobacco, sunflower, maize, bean, poplar and red pine (Turner 1969; Sanchez-Diaz and Kramer 1971; Teare and Kanemasu 1972; Turner and Begg 1973; Turner 1974) in the following respects.

The response of stomatal resistance to irradiance level was hyperbolic for young well-hydrated leaves.

The resistance of the abaxial leaf surface (to gaseous diffusion) was lower than that of the adaxial surface at all levels of irradiance in well-watered plants, at all three stages of plant development.

The diffusive resistance of both leaf surfaces was higher for older leaves than for younger leaves on well-watered plants.

The irradiance level at which the adaxial and abaxial stomata opened was higher for older leaves compared to younger leaves on well watered plants.

The resistance of the adaxial stomata was very high in leaves of well watered plants during pod filling.

The variation in diffusive resistance of adaxial stomata was greater than that of the abaxial stomata at the same irradiance level.

The diffusive resistance of both adaxial and abaxial stomata increased as the leaf wilted.

These effects, and mechanisms proposed for them, are discussed in the aforecited literature and so are not repeated here. The association between stomatal diffusive resistance and photosynthesis and their relationship to leaf age is examined in detail and discussed further in Section C and Appendix II. The behaviour of the stomata before and after the water stresses were applied requires mention.

The resistance of the adaxial stomata increased (despite a high irradiance level) before that of the abaxial stomata as the pre-flowering and flowering stresses developed. (Similar behaviour has been observed by Kanemasu and Tanner (1969) in *Phaseolus* leaves under stress.) The adaxial stomata are apparently more sensitive to declining leaf water status and so close at higher leaf water potentials. This could be an ecological adaptation to prevent excessive water loss at the beginning of stress without affecting photosynthesis as severely as would happen if the abaxial stomata also closed or partly closed. If the leaf is not rehydrated and the stress continues to develop the abaxial stomata also start to close. Also, some leaves dehydrated and died after stomatal closure, indicating that this mechanism is efficient for only temporary water deficits in leaves adapted to non-stress conditions. Upon rewatering, the resistance of the abaxial stomata declined before that of the adaxial stomata but it was usually some days before

resistances were again similar to those of well watered plants despite the leaves regaining turgidity. The lag in stomatal recovery and the greater sensitivity of the adaxial stomata to stresses have also been observed in other species (Kanemasu and Tanner 1969; Sanchez-Diaz and Kramer 1971; Beardsell and Cohen 1974). Water stress invokes hormonal and other biochemical changes in leaves (Kriedemann *et al.* 1972; Brady *et al.* 1974) and so a period of rehydration after stress is probably required to restore the usual hydrated chemistry and thus stomatal functioning (Kriedemann and Loveys 1974).

The measurement of stomatal diffusive resistance is a reliable method for measuring the physiological status of a leaf before water stress symptoms are visible and could be a useful technique in irrigation management. Although the adaxial stomata are the most sensitive to water deficits, their variability and sensitivity to other factors makes them less useful than the abaxial stomata for reliably indicating leaf water status.

Photosynthetic Behaviour

The net photosynthetic rates for leaves from the plants in the glasshouse were similar to some of the higher rates recorded for leaves of cv. Lee from field-grown plants (cf. Elmore *et al.* 1967; Dornhoff and Shibles 1970). Photosynthesis started to decline during pod filling at about the same time as leaf resistance increased. It is possible that the pods which did develop and mature provoked the reduction in F and the associated increase in r_s. Loveys and Kriedemann (1974) have shown that the removal of fruit in *Vitis* increased r_s and the concentration of abscisic acid in the leaves; a compound which appears to

influence stomatal behaviour during water stress and senescence (Jones and Mansfield 1970; Kriedemann *et al.* 1972). The photosynthetic and stomatal behaviour would also appear to support the hypothesis, which is introduced later, that an overall shortage of assimilate within the plant was not the primary cause of the low bean yields.

There was a large variation in the maximum rate of photosynthesis (F_{max}) between days. Variation in photosynthesis is also apparent in the literature: within experiments differences of the order of 50% in F_{max} have been reported between varieties, but between experiments there are differences of 100% within a variety (e.g. Curtis *et al.* 1969; Dreger *et al.* 1969; Dornhoff and Shibles 1970). Such variation is not unique to soybean (Loomis *et al.* 1971; Evans 1975). The variation observed in F in this experiment and that found in the literature may be related and so several variables were selected in order to determine the causes in the different situations. In this experiment, and others reported, leaf temperature was about the optimum for F and was always kept between 25° and 35°C where the Q_{10} is low (Hofstra and Hesketh 1969), irradiance was always saturating, and CO_2 concentration was usually ambient (310 $\pm$ 20 $\mu l\ l^{-1}$), so these factors did not appear to be responsible. Differences in photorespiration could not account for differences in F between some 50 varieties of soybean (Curtis *et al.* 1969; Dornhoff and Shibles 1970), and the chlorophyll concentration of a leaf has to be very low before it affects F (Gabrielsen 1948; Wolf 1965; Singh and Lal 1935) and so these factors are not considered to be responsible.

There is no conclusive evidence for soybean that F is dependent upon the stage of plant development *per se* except during senescence.

Koller *et al.* (1970) found that net assimilation rate (NAR) increased by 50% coincident with a rapid increase in seed weight, however, the effect may have been confused by the rapid abscission of lower leaves (Shibles *et al.* 1975). Sambo (1974) suggested that the increase in NAR which he observed in the varieties Lee and Harosoy at flowering and pod filling was a result of an increased efficiency of leaf photosynthesis. Dornhoff and Shibles (1970) and Ghorashy *et al.* (1971) have observed increases in F of individual leaves during pod filling but other factors such as leaf age or leaf position on the plants may have been partly or wholly responsible for the change. Maximum gross photosynthetic rates of single leaves may increase up the mainstem and are reached just before the leaf attains full expansion, after which rates decline rapidly (Kumura and Naniwa 1965). However, these authors used detached leaves exhibiting low gross photosynthetic rates which were not characteristic of field grown plants. Despite a lack of reliable data it is apparent that age and position can influence photosynthesis of soybean leaves.

The irradiance level under which leaves are grown also appears to influence F_{max} (Elmore *et al.* 1967; Bowes *et al.* 1972) as a result of an adaptive mechanism (Beuerlein and Pendleton 1971). However, Bowes and co-workers used detached leaves which may have resulted in the leaf exhibiting photosynthetic rates similar to those immediately prior to detachment, since there was no opportunity for stimulation of photosynthesis from the rest of the plant. Further, the acclimation observed by Beuerlein and Pendleton (1971) may have been a senescence effect provoked by unfavourable environmental conditions. The irradiance level during growth is the second factor which could introduce variation into measurements of F, but this is not unequivocal. An examination of daily

irradiation in the glasshouse showed no obvious correlation with daily F_{max} even when a time lag of several days was considered.

The principal factor causing variation in F in this experiment would appear to be the measurement of leaves of different ages and positions on the plants. This factor, and possibly light adaptation, could also have been responsible for some of the differences reported in photosynthesis of soybean leaves in the literature. Because of the marked lack of data concerning these factors for soybean, a study was initiated to clarify the effects of leaf age and position on the photosynthetic rate of intact leaves grown under different irradiance levels. This is described in Section B. The study is carried further in Section C where the effects of the reproductive stages and different sink sizes on photosynthetic rate is examined, together with other factors that limit or control photosynthesis.

Before lines can be bred for increased photosynthesis (and thus possibly increased yield, Shibles *et al.* 1975) genotypes with superior photosynthetic behaviour to present varieties must be identified (cf. Moss and Musgrave 1971; Loomis *et al.* 1971). This can only be done successfully if meaningful measurements are made that represent real differences and not artifacts of methodology.

An Explanation for the Low Bean Yields

The differences between bean yields were non-significant as a result of the high variation within treatments and the low overall yields. Only the stress during pod filling decreased yield compared to that of well-watered plants because it prevented further bean filling. The reasons for the low yields from all treatments (including well-watered plants) were not obvious.

The plants had the potential to produce about 500 g of beans (assuming a 40% harvest index cf. Laing 1974, p.40; or from data collected on day 109, the potential bean number per plant, 1950, times the expected bean weight, 0.2 g, yields 390 g). Overall, the low bean yields were attributable to low bean weights and the abortion of pods and possibly florets. This indicates a shortage of assimilate to these parts during the flowering and pod filling periods (see Review of Literature). However, as photosynthetic rates were not low during flowering and pod filling (cf. Dornhoff and Shibles 1970, cv. Lee), it is unlikely that there was an overall plant shortage of assimilate; especially one which could depress yields by 60-80%. Further, the dry weight of stems and leaves increased significantly from 26 days after pod filling commenced indicating translocation of assimilate to these parts. There is normally no increase in leaf or stem and petiole dry weight after this time (Koller *et al.* 1970; Hanway and Weber 1971). Therefore the low bean yields were not a result of an assimilate shortage within the plants but of the partitioning of assimilate between vegetative and reproductive sinks.

Vegetative growing points may have been dominant over the reproductive sinks for assimilate, or pods and florets aborted to an extent which permitted excess assimilate to go to vegetative parts. The latter course seems unlikely because the pods did not fill as well as would be expected if there was excess assimilate available to the beans (cf. bean weights in Section C and McAlister and Krober 1958). However, is it possible that factors causing abortion of reproductive sites could also adversely affect bean filling without affecting photosynthetic rates? This will now be considered.

Just prior to pods forming there was a rapid and substantial drop in total daily irradiance (by about 35%, Fig. A1) as a result of cloudy weather which continued for about five weeks. Shading (irradiance equivalent to less than 400 J cm^{-2} day^{-1}) by Mann and Jaworski (1970) after the time of pod formation, reduced pod fresh weight by 60% as a result of a 43% decrease in pod number per plant. These authors suggested that reduced assimilate production caused pod abscission. The decline in irradiance in my experiment was not as severe and the yield reduction was probably not related to reduced production of photosynthate. However, changes in irradiance may have contributed to pod abortion.

An excessive water supply to soybean plants causes accumulation of carbohydrate in leaves and stems, reduces absorption of nitrogen, and increases the shedding of flowers (Fukui and Ojima 1957). Further, Weber (1968) and Kerle (1947) observed that excess water during flowering and pod-set was associated with pod abortion despite the availability of assimilate. The vegetative growing points are physiologically dominant over the developing pods at this time and Weber suggested a moderate water stress at early pod-set may actually aid pod development. Similarly, in coffee (*Caffea* sp.) a water stress is required to break bud dormancy by removing a growth inhibitor (Alvim 1960). The soybean plants probably received excess water when the method of watering was changed at early pod-set as the bottom 7 cm of the bins was flooded. Further, relative humidities in the glasshouse were between 60 and 70% during the day and would have contributed to keeping the plants well hydrated. Thus, the pod abortion and poor bean filling were probably a result of the vegetative sinks maintaining their dominance for assimilate. This was possibly associated with an endogenous hormone im-

balance (cf. Stoy 1963). It is well recognised that roots supply hormones that control physiological processes in the tops (e.g. Wareing *et al.* 1968; Beever and Woolhouse 1974; Field and Jackson 1974) and thus a disturbance to root growth (e.g. by flooding) could result in changes in whole plant physiology.

The absence of leaf senescence may also be related to the poor pod-set and filling. Soybean leaves do not senesce if nearby buds or pods are removed (Hicks and Pendleton 1969; Section C) suggesting that a hormone is produced by mature pods to promote senescence of nearby leaves.

Some aspects of these results are examined further in Section D where plants were grown at different atmospheric humidities and with adequate water to determine, in part, the effect of hydration level of the plant on bean yield.

A Critique of Methodology

Several aspects of this experiment may validly be criticised. The first concerns the technique of stressing plants at one stage of growth (e.g. as used by Laing 1966, Boyer 1970 a,b) and relating the result to occurrences in the field or assuming the result is valid for all situations. Soybeans can adapt through growth and development to quite severe water deficits by the deposition of lipids on the leaf surface, by increasing the content of hemicellulose in the cell wall, and by producing a larger root system and a smaller leaf area (Clements 1937; Clark and Levitt 1956; Read and Bartlett 1972). Such a plant is more capable of retaining turgor, exploiting the soil volume, and maintaining water balance and adapting to the stress. Stressing well-

watered plants grown under controlled conditions almost certainly overestimates their reaction to a similar field stress since it does not permit the natural plasticity of the plant to develop.

Secondly, the absence of a measure of plant water status restricts the usefulness of the results and comparisons to other research. Measurements of plant or leaf water potential would have helped prevent the severe stress at pod filling which resulted in the plants dying prematurely. Visible wilting symptoms apparently occur at lower leaf water potentials as the leaf thickens and secondary cell wall thickening takes place. Thus, quantification of the level of stress applied is necessary.

Thirdly, the measurement of photosynthesis on single leaflets and the intention to relate this to plant behaviour was unsatisfactory. Changes in photosynthesis of single leaves under different physiological and environmental situations are not well understood in soybean. A better approach may have been to measure photosynthesis on whole plants which could then be related to yield reduction or stomatal behaviour, integrated over the whole plant. Even this technique could produce many problems. A slower imposition of the stresses (similar to the pre-flowering stress) would also have provided more useful information.

If the experiment were to be repeated to provide answers to the original questions it should be done in the field, the stresses applied more slowly, canopy photosynthesis and leaf water potentials should be measured, and possibly more treatments imposed with more replications of a treatment.

SECTION B. PHOTOSYNTHESIS AND EXPANSION OF LEAVES OF SOYBEAN GROWN IN TWO LIGHT REGIMES

Abstract

Net photosynthetic rates per unit area (F) were determined for soybean leaflets at different nodes on the mainstem from just after leaf emergence until senescence, on plants grown in a controlled environment cabinet or glasshouse. Final leaf area (A_{max}) and F_{max} increased up to the ninth node above the unifoliate node and the values for leaves on equivalent nodes were similar for glasshouse and cabinet grown plants. The times from leaf emergence to the attainment of A_{max} were also similar for cabinet and glasshouse plants, as were the times of leaf duration. F increased from leaf emergence, in a pattern similar to the increase in leaf area, until F_{max} was attained between the times of reaching 98% A_{max} (two days before A_{max}) and A_{max} in different leaflets and in one case six days after A_{max}. Photosynthesis of leaves from the growth cabinet usually declined quickly after reaching its maximum value. The implications of such patterns and variation in research requiring the determination of F or F_{max} are discussed.

INTRODUCTION

Photosynthetic rates of single leaves have often been determined when attempting to explain differences in yields of different cultivars of soybean (*Glycine max* (L.) Merrill) (Ojima and Kawashima 1968; Curtis *et al.* 1969; Dreger *et al.* 1969; Dornhoff and Shibles 1970), and for interpreting responses to treatments (e.g. Ghorashy *et al.* 1971; Brun and Cooper 1967). It is important to know the maximum rate of photosynthesis of an individual leaf and/or the leaf displaying the maximum rate before a higher photosynthetic rate can be claimed for a specific cultivar or treatment. Reported "maximum" rates of net photosynthesis for single leaves range from 9.6 (Wolf 1965) to 140 ng CO_2 cm^{-2} s^{-1} (Dornhoff and Shibles 1970; Ghorashy *et al.* 1971). Not all the variation can be explained by differences in the environmental conditions under which the plants were grown or measured. Two factors which have sometimes been ignored or treated superficially are leaf age and position.

Ojima *et al.* (1965) showed for only one soybean leaf that the net photosynthetic rate per unit area (F) reached a maximum (F_{max}) at the time the leaf reached maximum area (A_{max}), after which F declined slowly for several days and then rapidly. It can be shown from data of Kumura and Naniwa (1965), who used detached leaves from the mainstem of field grown soybeans, that the maximum gross photosynthetic rates were attained between 84 and 95% A_{max} and then declined within a few days. The gross photosynthetic rates obtained were very low and not representative of field grown plants. Dornhoff and Shibles (1974) have shown from data obtained for 11 days after full leaf expansion that F_{max} was reached between 2 and 6 days after A_{max}; however their conclusions are equivocal as the results were variable and no statistical data are presented.

The relationship between net photosynthesis, area and age was

investigated for different leaves on the mainstem of developing soybean plants grown under two contrasting light regimes: in a glasshouse and a growth cabinet.

MATERIALS AND METHODS

Plant Culture

Experiment 1 - Growth Cabinet

Graded (220-230 mg), pre-germinated seeds of soybean cv. Lee were sown without *Rhizobium* inoculation in a fertile sandy-loam soil in 0.05 m^3 bins in a controlled environment growth cabinet. Temperatures during the light and dark periods were 30 $\pm$ 0.5°C and 25 $\pm$ 0.5°C respectively; vapour pressures were 36 mb and 22 mb respectively. The irradiant flux density 0.5 m below the VHO CW fluorescent/incandescent light source was 590 µE m^{-2} s^{-1} (400-700 nm) or about 70 J cm^{-2} h^{-1} (short-wave 400-1100 nm) at the finish of the experiment. The light-bank was moved upwards as the plants grew so that the top leaves were under constant irradiance. The initial photoperiod was 15 h and was reduced to 12 h 24 days after seedling emergence. Inflorescences were visible in the leaf axils 11 days later, and pod filling commenced another 24 days later. A continuous subterranean watering system prevented water deficits, and the plants received a complete nutrient solution fortnightly.

Experiment 2 - Glasshouse

Culture conditions were the same as for experiment 1 except that the plants were grown in a temperature-controlled glasshouse from February to May at Canberra. The natural photoperiod was extended to 14 h by incandescent lamps. Inflorescences were visible 27 days after seedling emergence, and pod filling commenced 24 days later. The mean daily short-wave (400-1100 nm) irradiant flux density inside the glasshouse for the

months February to May was 1505 ± 91, 1140 ± 67, 557 ± 65 and 524 ± 35 J cm^{-2} respectively (measured with a silicon cell integrating pyranometer). The maximum irradiant flux density during these months was approximately 2000, 1900, 1400 and 1300 μE m^{-2} s^{-1} respectively (measured with a Lambda Instruments quantum sensor).

Measurement of Gas Exchange and Stomatal Diffusion Resistance

Net photosynthetic rates of terminal leaflets were measured in an open system using an URAS 2 infra-red gas analyser calibrated with gas mixing pumps. The apparatus is described in detail in Appendix I. The leaflet temperature was maintained at 29.9 ± 0.1°C; the vapour pressure deficit was 15 mb; the CO_2 concentration of the inlet air varied between 320 and 340 μl l^{-1}; the CO_2 depression was <25 μl l^{-1}. The light source provided an irradiant flux density (400-700 nm) of >1700 μE m^{-2} s^{-1} on the adaxial leaf surface. The leaf chamber, light source and experimental plant were in a growth cabinet kept at 30°C. F usually stabilized quickly and the maximum rate within one hour was used; the leaf area was then determined using a leaf imprint made on studio proof photographic paper and then measuring the area of the print with an electronic planimeter.

Two plants were selected from each light regime and used for all photosynthetic measurements. F was determined for leaves on nodes 3, 5 and 9 (terminal) up the mainstem of the glasshouse-grown plants, and for leaves on nodes 1, 3, 5, 7 and 9 from the cabinet-grown plants (the unifoliate leaf node was 0 and the terminal node 19).

Stomatal resistance to water vapour diffusion (r_s) was determined each week with an aspirated diffusion porometer (Byrne *et al.* 1970) on leaves on nodes 1, 3, 5, 7 and 9 from two plants from the growth cabinet under the *in situ* irradiance level. Leaf areas were determined twice weekly.

RESULTS AND DISCUSSION

Individual data points of A and F from both replicates for plants grown in the cabinet are shown in Figs. B1 and B2. The leaf expansion curves for each pair of leaves were matched for development by fitting Richards type functions (Richards 1959) which enabled the use of a common time base for F. Each successive leaf up the mainstem reached a larger A_{max} and F_{max} than that below it. F_{max} was attained at between 98 and 100% A_{max} (indicated by arrows in Fig. B2) and then declined for leaves 1, 3, 5, 7 and 9. The decline in F of leaf 9 took considerably longer than for lower leaves. This maintenance of F may have been a result of the higher irradiance on leaf 9 during its senescent phase (e.g. on day 47 the irradiance levels on the adaxial surfaces of leaves 7 and 9 were 70 and 130 $\mu E\ m^{-2}\ s^{-1}$ respectively) or of a stimulation of F by the flowers and developing pods, cf. Zhailibaev and Khasenov (1966) and Beever and Woolhouse (1974). The increase and subsequent decrease in F were associated with changes in stomatal resistance to water vapour diffusion (Fig. B3). The stomatal resistance appeared to decline as the leaf expanded and reached a minimum value at about the time A_{max} was reached, after which r_s again increased. This increase may not be entirely a result of leaf ageing but could involve stomatal closure as a direct consequence of lower irradiance levels. This relationship between F and r_s is examined in more detail in subsequent sections of this thesis.

Leaf expansion data from the glasshouse plants were also treated as described previously and normalised to $\bar{A}_{max}$, and are shown with photosynthesis data in Fig. B4. Each point is a mean value of F as both replicates were measured on the same day. F_{max} occurred very close to the time A_{max} was attained for leaves 3 and 5. F_{max} of leaf 9 occurred some six days after the attainment of A_{max} and was maintained for eight days

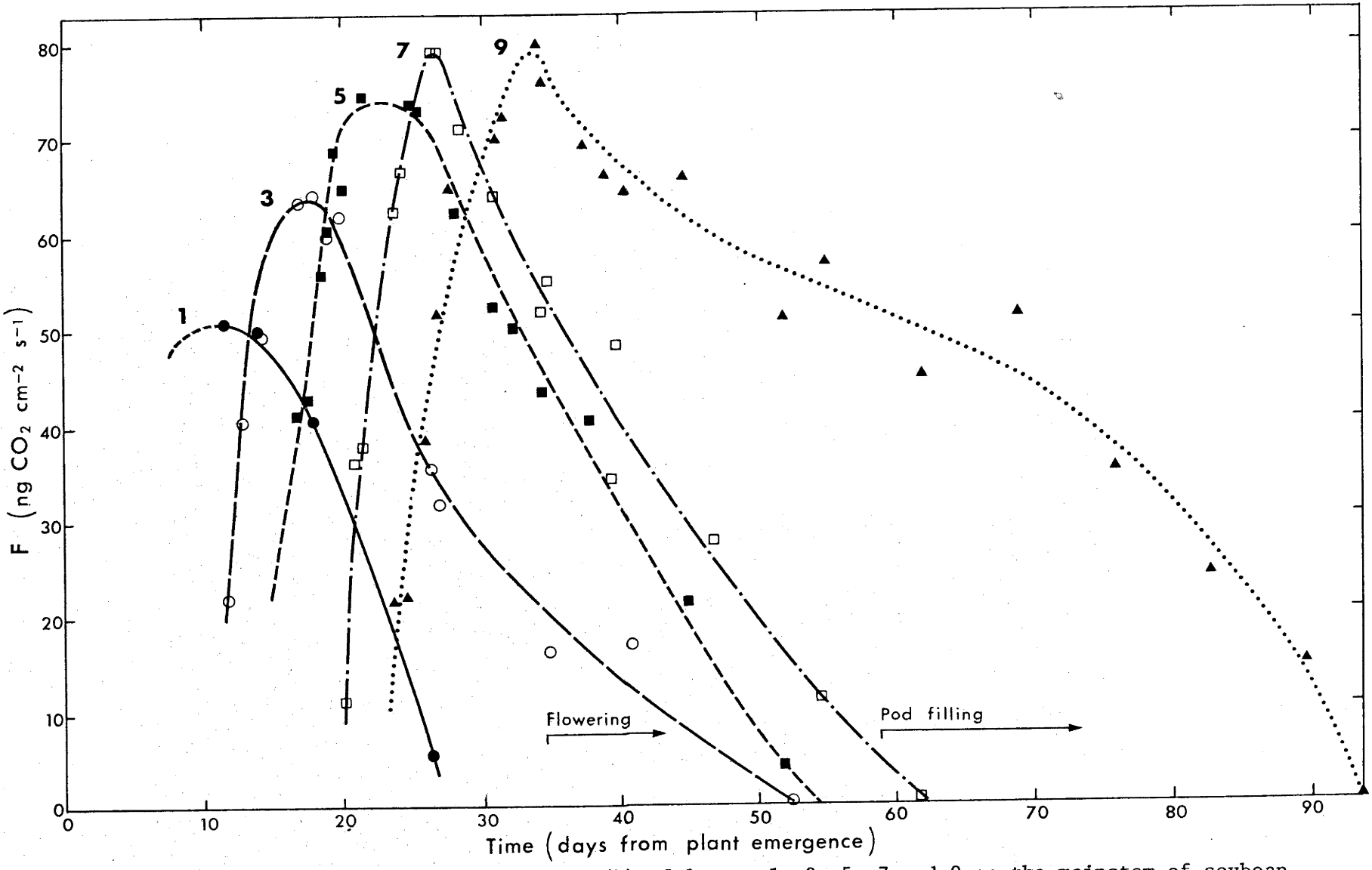

Fig. B1. Net photosynthetic rates (F) of leaves 1, 3, 5, 7 and 9 on the mainstem of soybean plants from a controlled environment cabinet as a function of number of days after seedling emergence; data from two plants

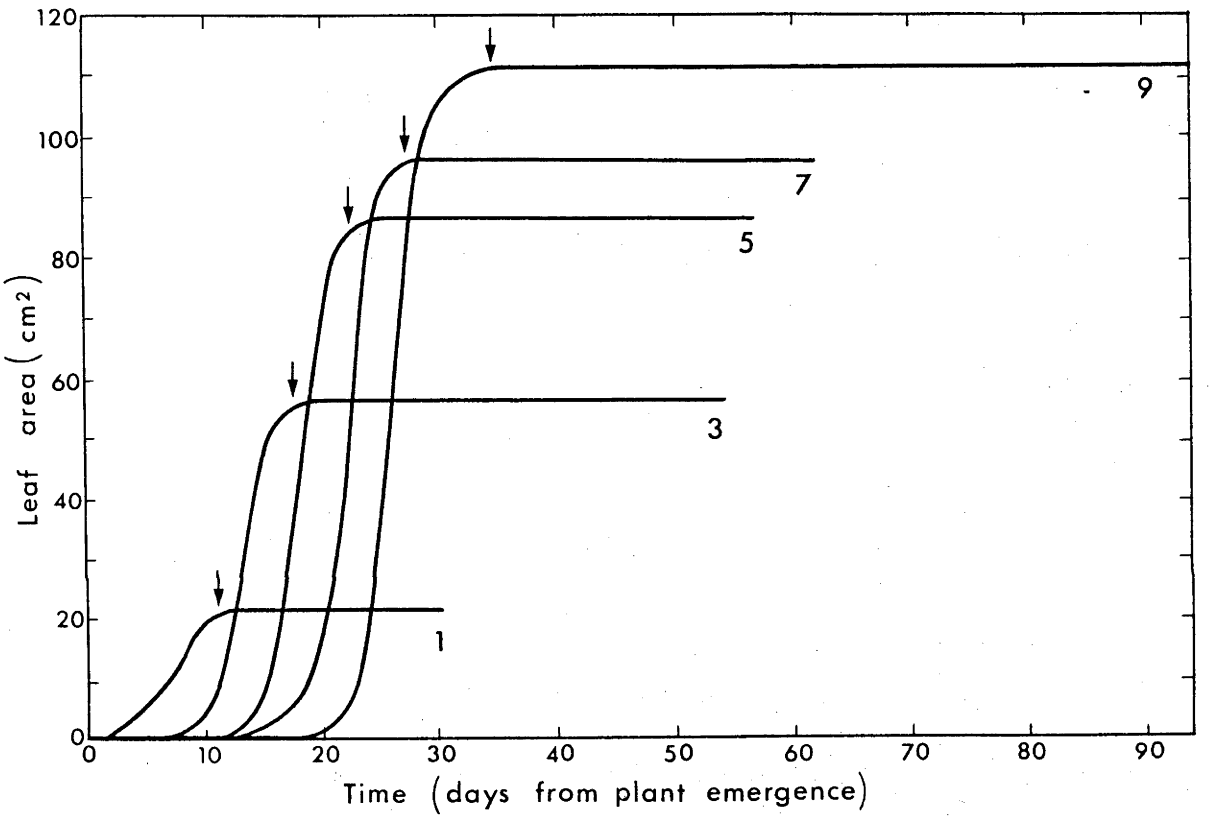

Fig. B2. Areas of leaves 1, 3, 5, 7 and 9 on the mainstem of soybean plants from a controlled environment cabinet as a function of the number of days after seedling emergence; arrows indicate the timing of F_{max}; mean data from two plants

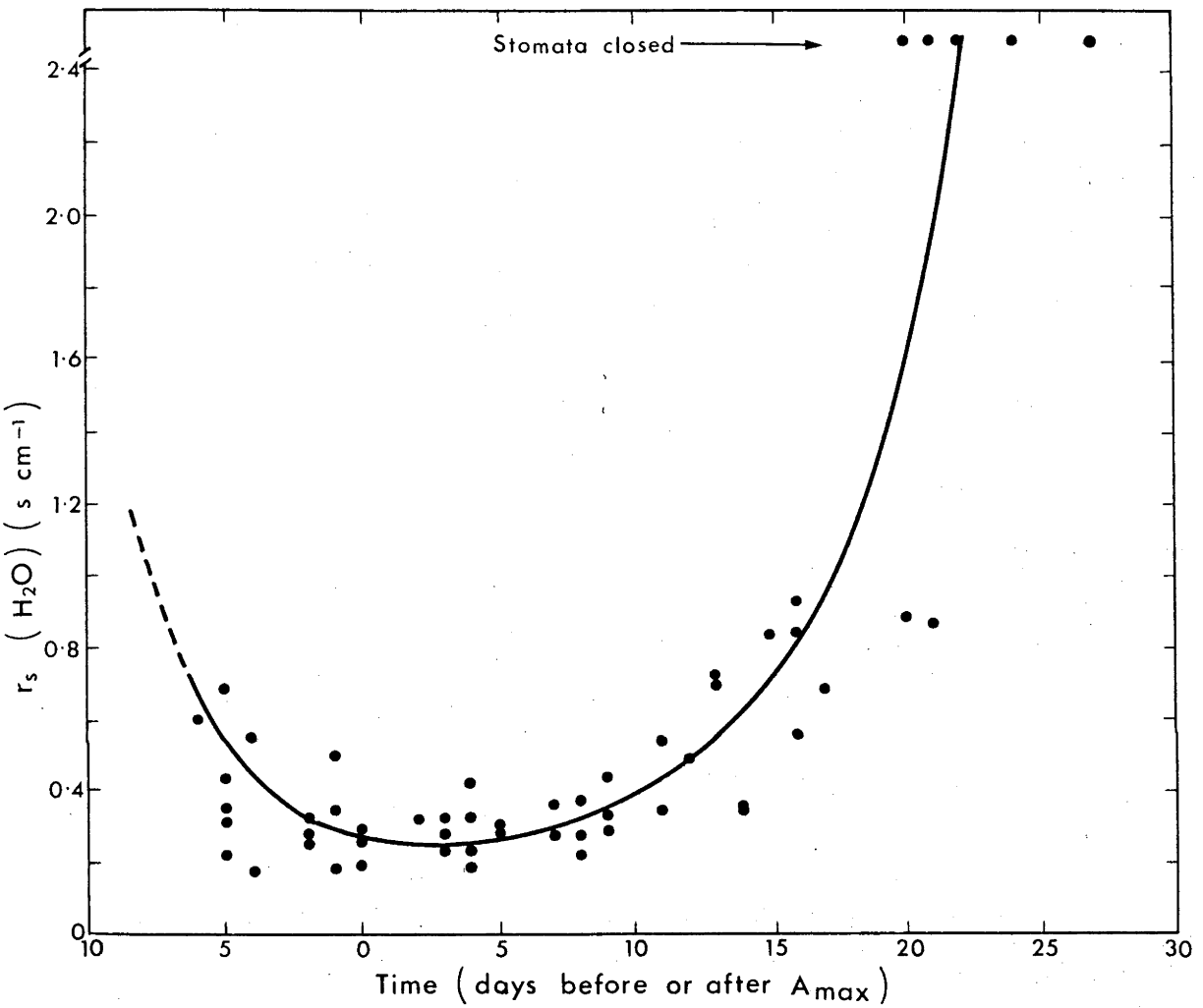

Fig. B3. Stomatal resistance to diffusion of water vapour (r_s) for the abaxial surface of leaves 1, 3, 5, 7 and 9 on the mainstem of soybean plants from a controlled environment cabinet as a function of days before or after A_{max}; data from two plants and measured under ambient irradiance

before declining. The depressions in F about the middle life of these leaves cannot be explained by daily variations in sunlight intensity, but may be a result of changing hormone levels (cf. Beever and Woolhouse 1974; Loveys and Kriedemann 1974) or the changing requirement for assimilates during flowering and pod filling, and during senescence of lower leaves. The second peak in F was never higher than the first. The second peak was not exhibited by leaves from the growth cabinet; possibly any internal stimulation of F was depressed by the decreasing irradiance in the cabinet, although there appeared to be little effect of light regime on leaf duration. Further experiments to help elucidate the causes of these peaks were carried out and are described in Section C.

The F_{max} of leaves from the glasshouse was similar to that of the equivalent leaf from the growth cabinet. Bowes *et al.* (1972) found that detached soybean leaves exhibited higher F_{max} when previously grown under higher irradiance, but Doley and Trivett (1974) found no difference in F_{max} for intact *Astrebla* leaves grown in a glasshouse or growth cabinet.

The attainment of F_{max} just before or just after the leaflet lamina becomes fully expanded would appear to be a characteristic of soybean. A similar pattern has been observed in grape (Kriedemann *et al.* 1970) and cottonwood (Isebrands and Larson 1973), whereas in tobacco (Rawson and Hackett 1974), capsicum (Steer 1972) and cucumber (Hopkinson 1964) F_{max} occurred between 20 and 50% A_{max}. It is advantageous for soybean, whose growth and yield may be source limited (Hardman and Brun 1971; Literature Review, this thesis) to have F_{max} coincide with A_{max}, as it appears from the limited data available that F decreases rapidly where F_{max} occurs earlier in leaf expansion (and cf. the effect of a sink limitation on F in tobacco, Appendix II).

Thus, maximum photosynthetic rates for soybean leaves varied

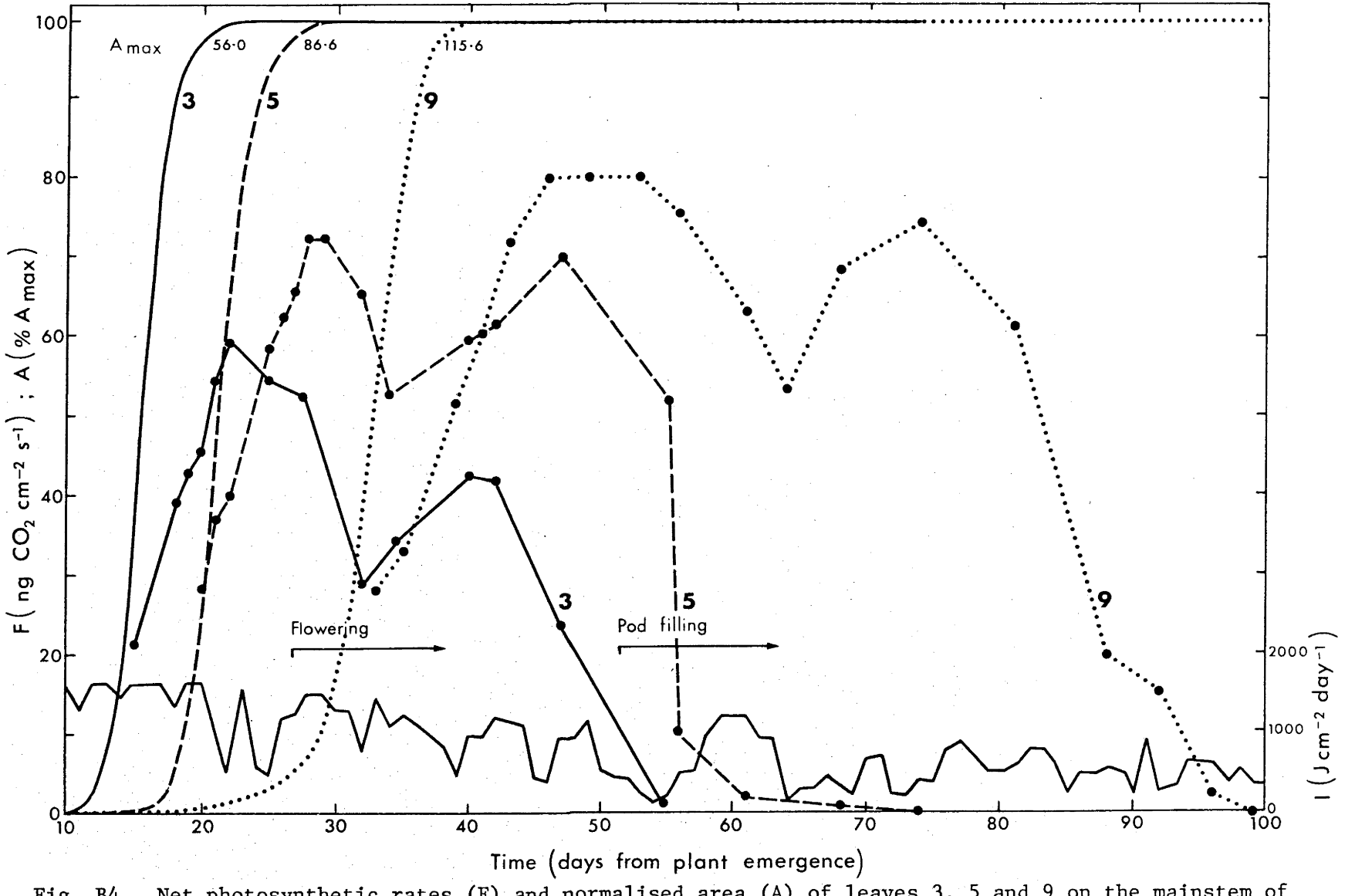

Fig. B4. Net photosynthetic rates (F) and normalised area (A) of leaves 3, 5 and 9 on the mainstem of soybean plants from a glasshouse as a function of the number of days after seedling emergence (mean data from two plants) and total daily shortwave irradiant flux (400-1100 nm) in the glasshouse

with leaf position on the plant, and were reached between 98% A_{max} and six days after A_{max}. F should be measured at least during this period in varietal evaluation and other work relying on estimates of F_{max}. Complementary leaf expansion data are also required as the maximum individual leaf area changes up the mainstem. Ideally, F should be integrated over the life of the leaf, as spot measurements of F have little practical relevance to total photosynthate production or availability. Low correlations have been found between bean yields and maximum net photosynthesis of leaves in soybean cultivars (Curtis *et al.* 1969). A higher correlation has been found between yield and the length of the pod filling period and total leaf area (Dornhoff and Shibles 1970). Cultivars with high leaf photosynthetic rates may have a post-flowering period that is too short to permit adequate pod filling, whereas cultivars which exhibit lower photosynthesis rates may have longer pod filling periods or some other character conducive to greater total production of assimilate.

SECTION C. PHOTOSYNTHESIS AND TRANSPIRATION OF EXPANDING AND SENESCING LEAVES OF SOYBEAN

Abstract

The history of net photosynthesis and transpiration per unit leaf area was determined for intact soybean leaves from their unfolding to senescence during flowering and pod filling on untreated and partially depodded plants growing in a glasshouse. Leaf diffusive resistances to carbon dioxide were calculated and a water use efficiency parameter was derived (net mass of carbon dioxide fixed per unit mass of water transpired per millibar vapour pressure deficit).

Net photosynthesis and transpiration behaved similarly through all stages of leaf development. A number of peaks were evident in these parameters. The first was associated with leaf expansion and occurred when the leaf reached its maximum area. The second peak coincided with flowering of the plant and later peaks occurred during pod filling. Stomatal and mesophyll (residual) resistances also exhibited similar behaviour during the life of the leaf; the possible causes of this linkage are discussed. Water use efficiency increased rapidly up to the time of full lamina expansion, then increased slowly or remained stable until leaf senescence approached, when the efficiency declined.

Net photosynthesis and transpiration of leaves were very similar in both podded and partially depodded plants. It appears that to prevent a shortage of assimilate during flowering and pod filling, photosynthesis may be maintained or increased in some leaves and the response is not related to the number of pods available for filling. The increases in photosynthesis were correlated with both higher stomatal and mesophyll conductances. Mechanisms by which the plant may control leaf photosynthesis are discussed.

INTRODUCTION

It has been shown that net photosynthetic rate per unit area of soybean leaves reached a peak value at the same time as the lamina reached full expansion. In leaves from plants grown in the glasshouse, photosynthesis declined after the first peak, but then increased again to a second peak before falling as the leaves senesced. It was suggested that this later peak in photosynthesis was related to the reproductive stage of growth of the plants. Dornhoff and Shibles (1970) and Ghorashy *et al.* (1971) have measured higher photosynthetic rates in soybean leaves during pod filling compared to those at earlier stages of growth but these increases may have been confounded by the effects of leaf age or position. In this section, the photosynthetic histories of intact soybean leaves which developed during flowering and pod filling are presented. The aim of the experiment was to determine whether the timing and maintenance of maximum photosynthetic rates in leaves can be influenced by the reproductive stages of plant growth. In addition, some pods were removed from some of the plants to determine if the rate and pattern of photosynthesis with time could be altered by the absence of a sink.

Previous work has also shown that the increase and subsequent decline in photosynthesis as a soybean leaf expanded and senesced was associated with changes in the stomatal resistance to CO_2 diffusion (r_s). However, the mesophyll (or residual) resistance to CO_2 diffusion (r_m) is greater than the stomatal resistance in soybean leaves (Dornhoff and Shibles 1970; Beardsell *et al.* 1973b). It was desirable to determine if this relationship changed during the life of the leaf and thus determine the relative importance of these two resistances in control-

ling the diffusion of CO_2 from the atmosphere to the carboxylation site. Therefore a diffusion resistance analysis is presented for leaves of soybean grown in a glasshouse. The analysis was also carried out on the leaves of plants which were partially depodded in order to elucidate the reason for changes in photosynthesis as a result of depodding.

MATERIALS AND METHODS

Plant Culture

Experiment 1 - Leaf Age and Plant Phenology

Graded, pre-germinated seeds of soybean (*Glycine max* (L.) Merrill) cv. Lee were grown individually in 0.01 m^3 buckets containing a mixture of 1 part sand to 2 parts 'compost', without *Rhizobium* inoculation. Glasshouse temperatures were 30 $\pm$ 1°C for 12 h during the day and 25 $\pm$ 1°C during the night. Water was supplied continuously by a drip system and slow release tablets positioned below the soil surface provided nutrients. Three 400 W mercury vapour lamps placed 0.5 m above the plants minimized variation in irradiance during cloudy periods. The maximum photosynthetic (400-700 nm) quantum fluxes at the top of the plants were 2200 $\mu E\ m^{-2}\ s^{-1}$. As the lights provided a 12 h photoperiod, the plants flowered and filled pods.

Net photosynthesis and transpiration rates were measured on leaf 4 (unifoliate leaf is 0) on the main stem of four plants from just after the leaf unfolded until it commenced to senesce. All replicates were measured daily at first but less frequently as the leaves aged. Self shading was minimal and the measured leaves were oriented towards the north. At maturity the main stem had nine nodes above the node of the unifoliate leaf.

Experiment 2 - Leaf Age and Partial Depodding

Soybean plants were grown individually in 0.05 m^3 bins under conditions similar to experiment 1, except that supplementary irradiation was not provided in the glasshouse. Water was supplied by a continuous subterranean system which prevented a water deficit or an excess and the plants received a complete nutrient solution fortnightly. Four similar plants were selected at flowering and thereafter all flowers or developing pods were removed from the main stem of two of the plants. Pods were left on the branches. Each plant developed 15 nodes (above the node of the unifoliate leaf), and branches developed on the lower eight nodes. Net photosynthesis and transpiration rates were measured on leaf 14 on the main stem from its unfolding during flowering until its death. All plants were measured daily at first but less frequently as the leaves aged.

The mean daily total shortwave (400-1100 nm) irradiant flux in the glasshouse for seven-day periods from the commencement of leaf measurements in experiment 2 was 786 $\pm$ 196, 1548 $\pm$ 105, 995 $\pm$ 188, 1495 $\pm$ 100, 1225 $\pm$ 171, 1667 $\pm$ 97, 1605 $\pm$ 122, 1384 $\pm$ 81 and 1703 $\pm$ 115 J cm^{-2}; experiment 1 commenced during the third seven-day period.

Gas Exchange Measurements

The open gas system used to measure rates of net photosynthesis and transpiration in intact leaves is described in Appendix 1, but with the following variations. The entire terminal leaflet of the trifoliolate leaf was measured at 28.7 $\pm$ 0.1°C leaf temperature, 27.4 $\pm$ 0.1°C air temperature, 16.4 $\pm$ 0.1 mb vapour pressure deficit (VPD) of air surrounding the leaflet, and a photosynthetic quantum flux of 1800

$\mu E\ m^{-2}\ s^{-1}$. The mean CO_2 concentration of the inlet air was 325 $\pm$ 1 $\mu l\ l^{-1}$ and CO_2 depletion across the chamber was kept below 20 $\mu l\ l^{-1}$. (These mean values $\pm$ standard errors were derived from all measurements presented in this section). An A.D.C. (Analytical Development Co. Ltd., U.K.) infra-red gas analyser was used for differential CO_2 measurements. The plant was removed from the glasshouse and the leaflet was immediately sealed into the assimilation chamber. Maximum exchange rates were attained usually within 15 minutes and CO_2 compensation points were attained within 45 minutes, but older leaves took longer. All measurements were done between 0930 and 1330 h.

Calculation of Leaf Diffusive Resistances to CO_2

Leaf diffusive resistances were calculated as described in Appendix I; the decrease in CO_2 compensation point as the leaf expanded was less pronounced in soybean than in tobacco (Appendix II), and a mean of all measurements, 38 $\pm$ 1 $\mu l\ l^{-1}$, was used. Boundary layer resistance (r_a, $s\ cm^{-1}$) was adequately described by the function $r_a = 0.16\ \ln (1 + A)$, $r^2 = 0.998$ for leaf areas (A) less than 100 cm^2.

RESULTS

Leaf Age and Gas Exchange (Experiment 1)

The increase in leaf area with time was described by fitting a Richards curve (Richards 1959) to the mean data, r = 0.999 (Fig. C1). The mean net photosynthetic rate per unit area (F) of leaf four increased initially and reached a first peak value at the same time as A_{max} was attained on about day nine. A second higher peak occurred 11 days later (F on day 20 was significantly greater than F on day 16, $P < 0.05$) and

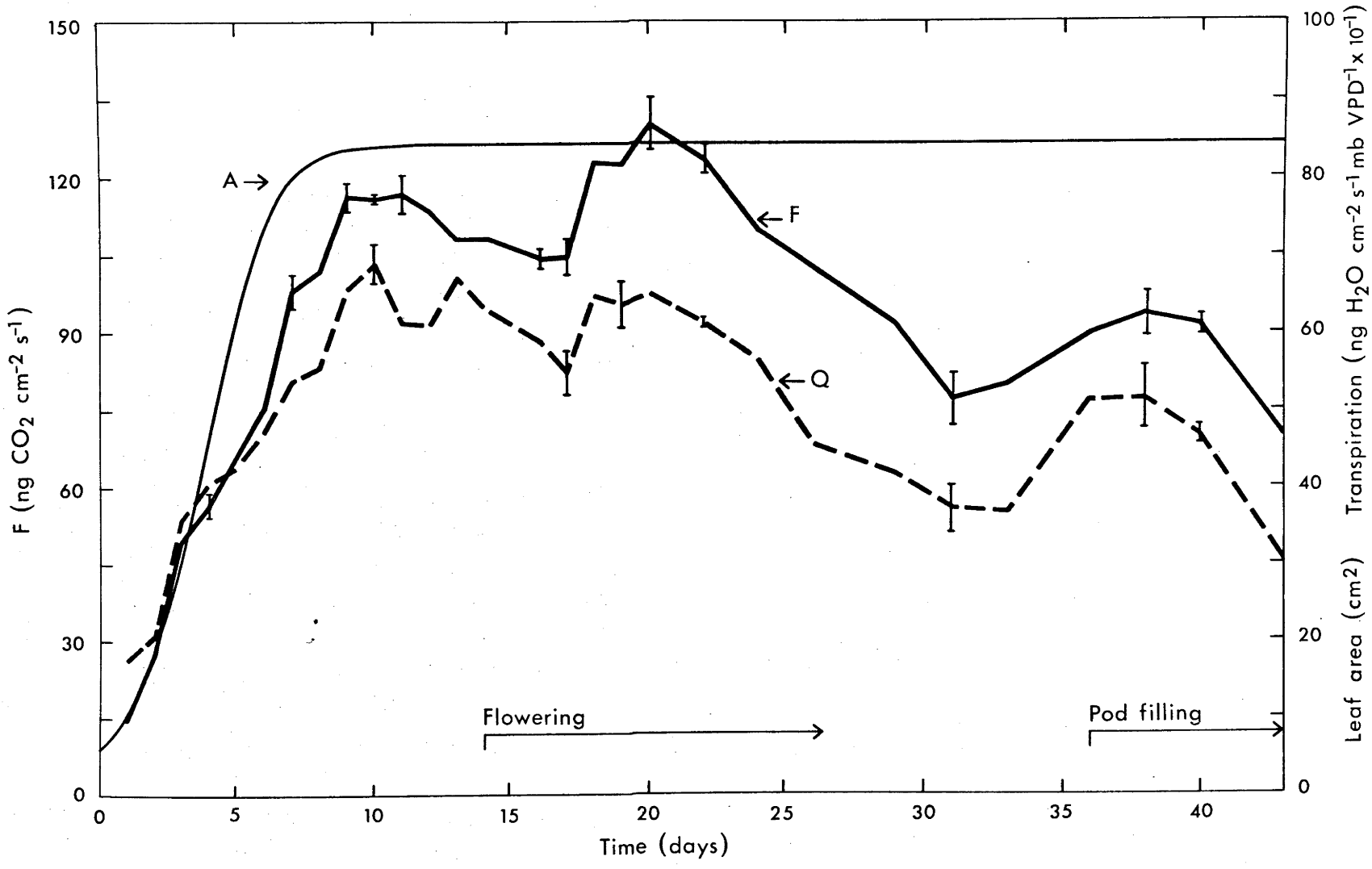

Fig. C1. Net photosynthesis (F) and transpiration (Q) per unit area, and leaf area (A) for the fourth leaf on the main stem of a soybean plant (mean of four plants); bars represent ± standard error

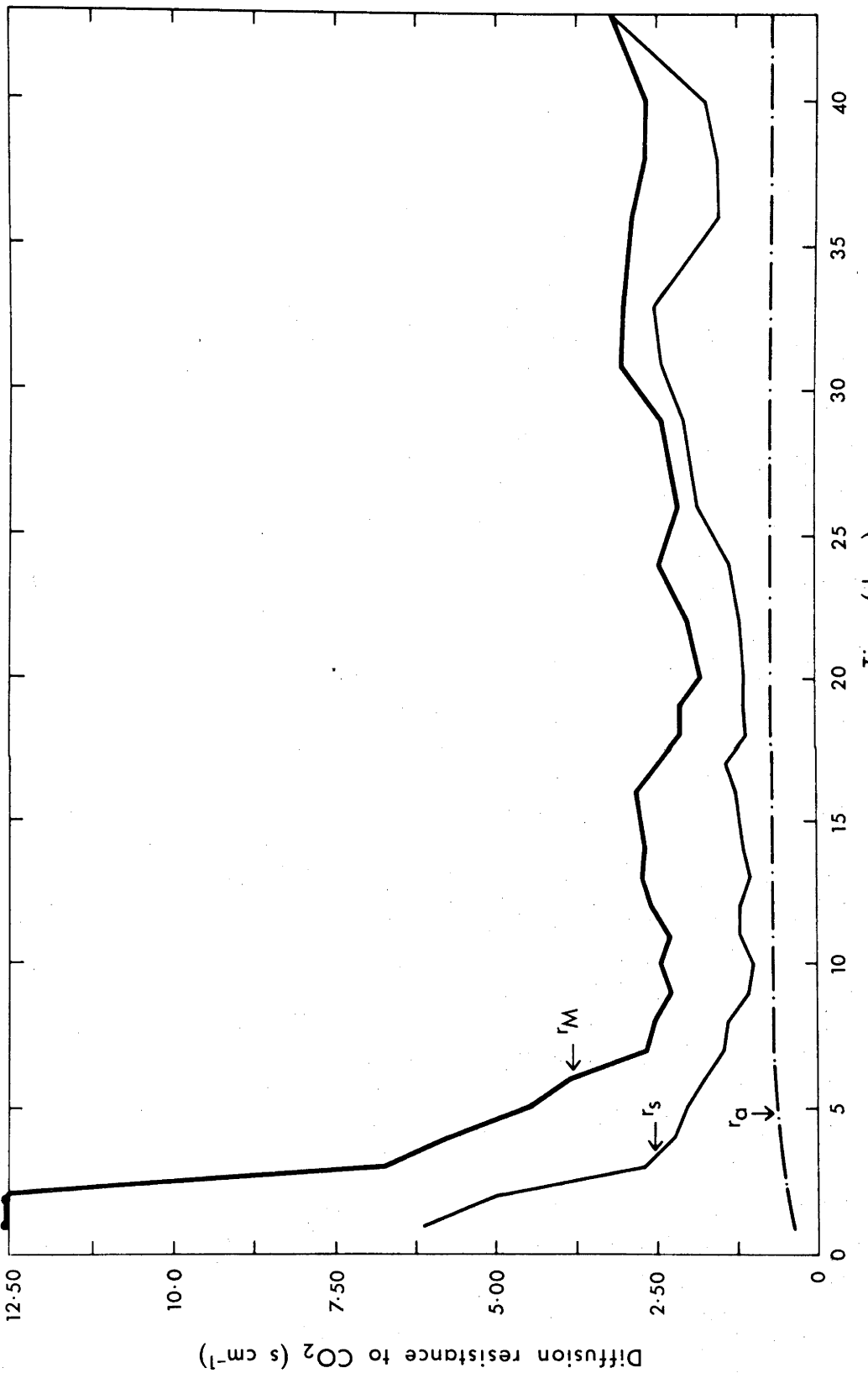

Fig. C2. Boundary layer resistance (r_a), stomatal resistance (r_s) and mesophyll or residual resistance (r_M) to CO_2 for the fourth leaf on the main stem of a soybean plant (mean of four plants)

there was a third peak 18 days after the second (F on days 38 and 40 was not significantly greater than F on day 31, P >0.10). After this time F declined again and measurements soon ceased as two leaves were damaged. Petals were first visible on the plants on day 14 and pod filling commenced about day 36 (both indicated on Fig. C1).

The net mass flux of water vapour per unit leaf area (Q) expressed per mb VPD was similar to the pattern exhibited in F (Fig. C1). This relationship is also clear from the parallelism between stomatal resistance (r_s) and mesophyll resistance (r_M) to CO_2 transfer shown in Fig. C2 and from comparable results for leaves from experiment 2 (Fig. C5). The r_M was very high when the leaf was young, but both r_s and r_M declined as the leaf expanded. The resistances were relatively stable between the time of reaching 95% A_{max} and the start of leaf senescence. r_M was always higher than r_s. Relative changes in the two resistances (ratio r_M:r_s) were not constant (Fig. C3), the ratio falling from about 5 near the time of leaf emergence to about 1.5 after the time A_{max} was reached. Also shown in Fig. C3 is the water use efficiency (ω, the mass of CO_2 assimilated per unit mass of water transpired, expressed per mb VPD in order to account for small fluctuations in humidity of the air in the chamber and to permit comparisons with other research) which showed a pattern similar to that of the ratio r_M:r_s. ω provides a more reliable measure of the relationship between r_M and r_s because the resistance ratio accentuates any error resulting from the method of calculating the resistances. For example, r_M is derived by difference between the total resistance and (r_a + r_s) and any errors in the latter term (there are several possible sources in the method used) are reflected in r_M with the same magnitude but opposite sign. As the leaf expanded ω increased

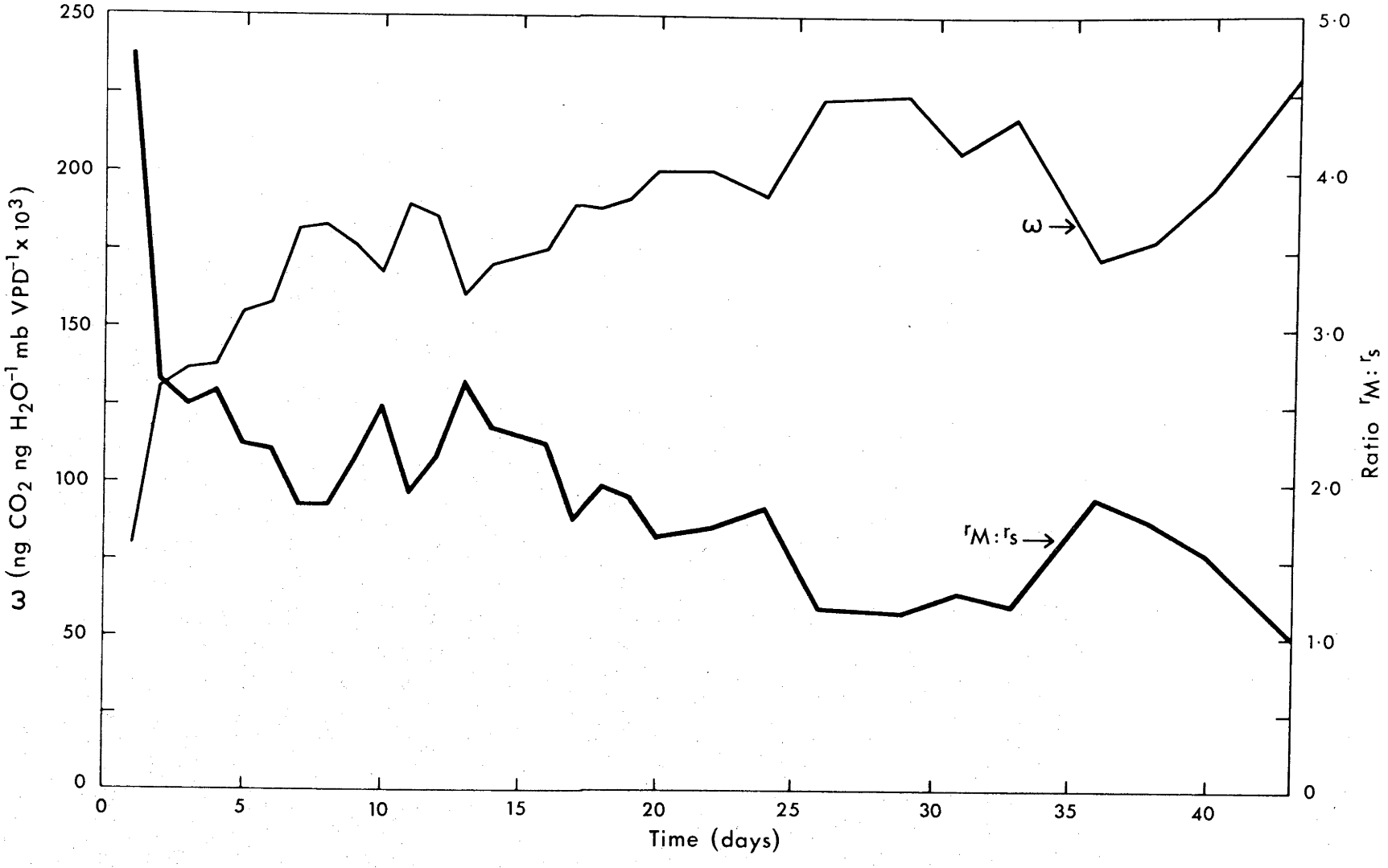

Fig. C3. The ratio of mesophyll resistance to stomatal resistance (r_M: r_s) and the water use efficiency (ω, see text) for the fourth leaf on the main stem of a soybean plant (mean of four plants)

rapidly and continued to increase slowly until just prior to the third peak in F. The relative stability of ω reflects the parallelism between F and Q during the life of the leaf (also cf. Fig. C6).

Partial Depodding and Gas Exchange (Experiment 2)

Mean photosynthetic rates for leaves frompodded and partially depodded plants are shown in Fig. C4. Q is not shown because it exhibited almost identical behaviour to F in both treatments. Flowers were present on all nodes of the plants three days before measurements commenced and pod filling commenced about day 14. The second peaks in F about day 32 (significantly greater than F on day 26, $P < 0.05$) occurred at a time when the pods were filling rapidly and the lower leaves were senescing in the podded plants. The peak associated with flowering observed in experiment 1 was not observed here because the measured leaves started to expand during flowering. The total daily shortwave irradiance in the glasshouse is also shown in Fig. C4. There appeared to be no correlation between irradiance, temperature, or nutrient supply with the fluctuations observed in F in either experiment, a finding previously demonstrated (Section B). Leaves from podded and partially depodded plants showed characteristics described in experiment 1. These were:

1. That the first peak in F was close to the time that A_{max} was attained;
2. The parallelism present in the behaviour of F and Q with time;
3. There being more than one peak in F and Q;
4. The close relationship between r_s and r_M (Fig. C5);

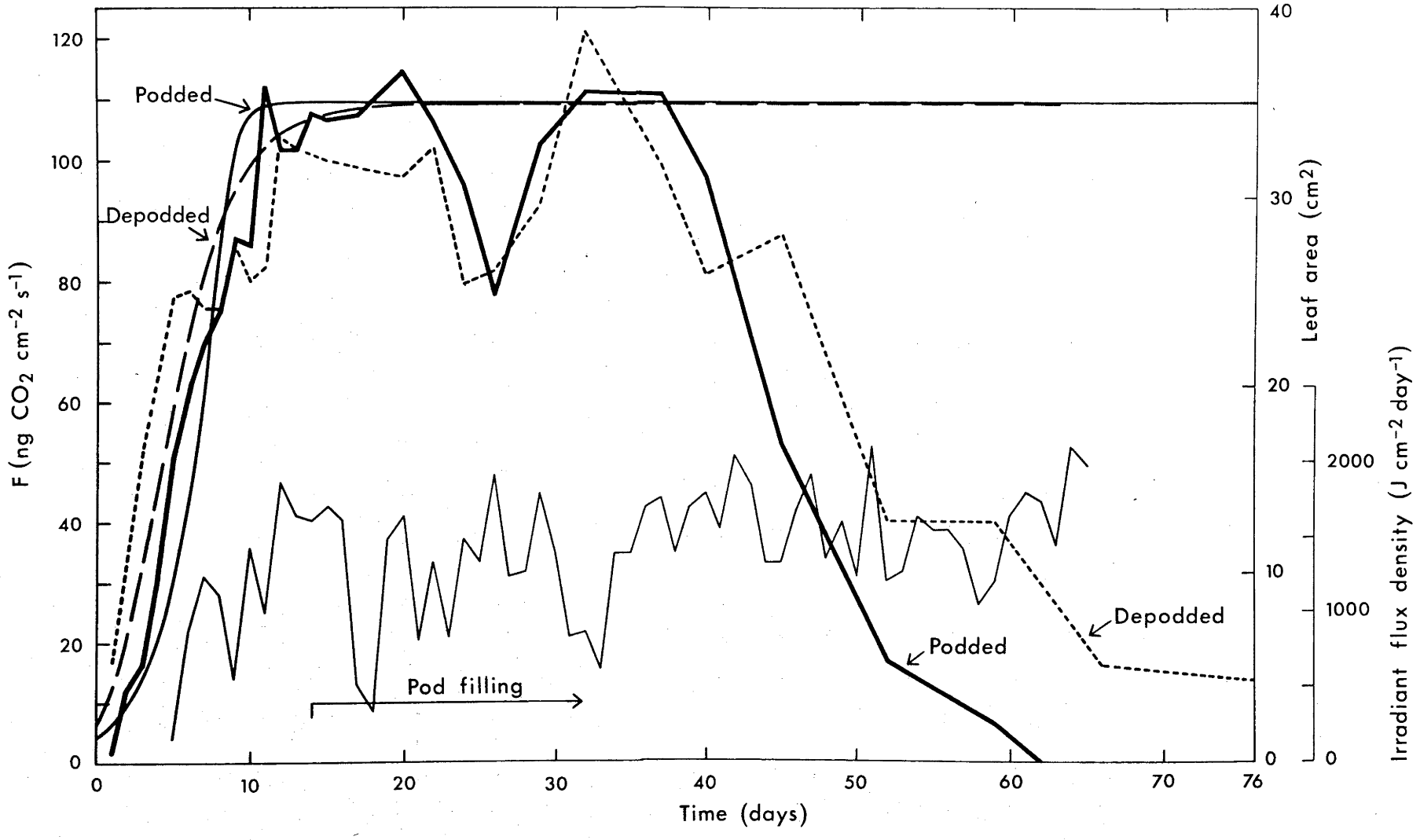

Fig. C4. Area and net photosynthesis (F) for leaves on the main stem of partially depodded and untreated soybean plants, from leaf emergence (mean of two plants), and daily shortwave irradiation in the glasshouse

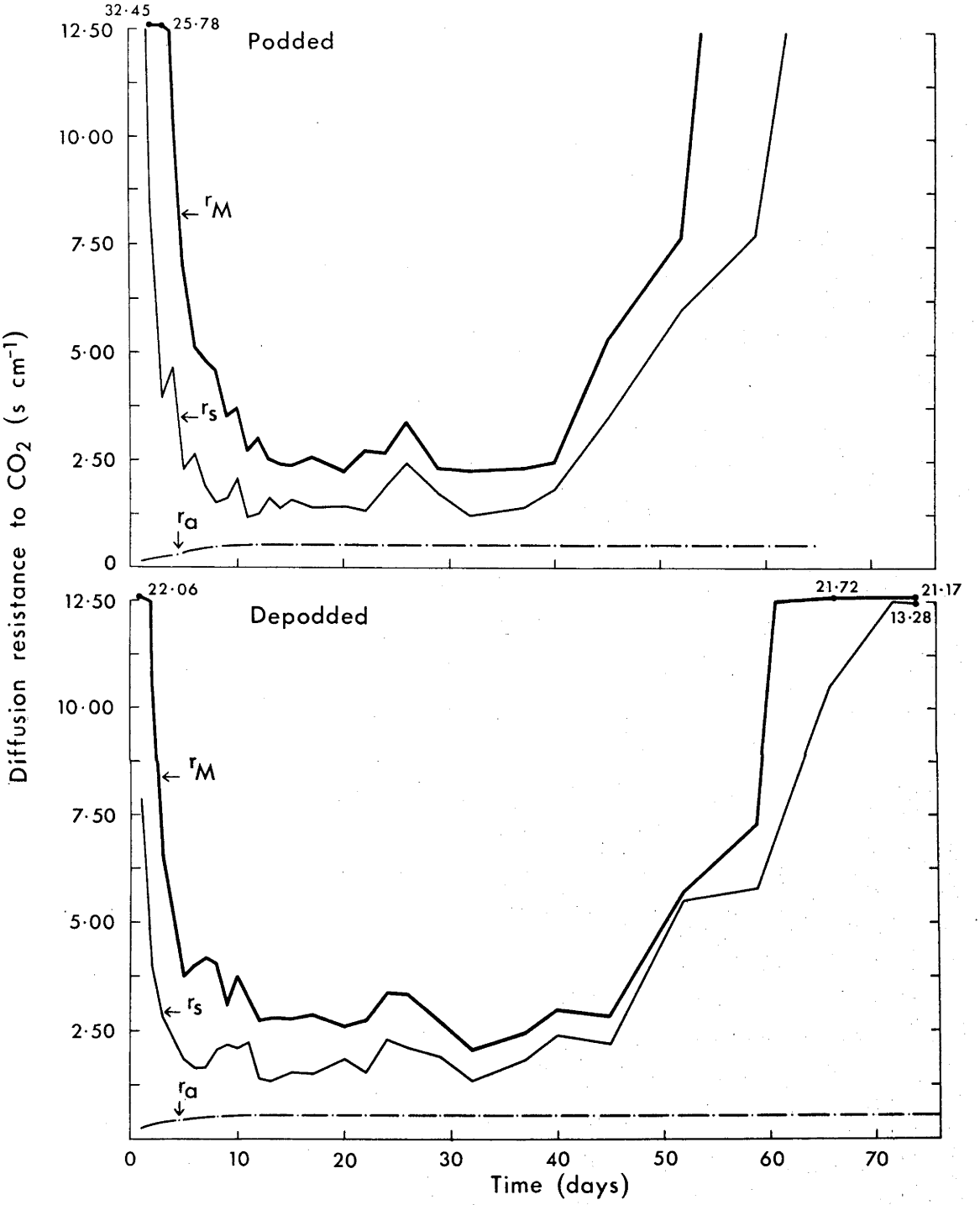

Fig. C5. Boundary layer resistance (r_a), stomatal resistance (r_s) and mesophyll or residual resistance (r_M) to CO_2 for leaves on the main stem of partially depodded and untreated soybean plants, from leaf emergence (mean of two plants)

5. That r_M was consistently greater than r_s;
6. The slow increase and stability of ω after the time A_{max} was attained and before leaf senescence commenced (Fig. C6).

The trends of F and Q with time were almost identical for podded and partially depodded plants. Partial depodding did not affect the magnitude of F, Q, r_s, r_M or ω. Although there were 6 nodes below node 14 (the measured leaf) on the depodded plants with no beans or branches (while there were 62 pods with 140 beans on the top 8 nodes of the podded plants) the only obvious effect of partial depodding on the measured leaf was to extend its life by maintaining F and Q at a low positive rate. All leaves on the main stem of the podded plant senesced normally whereas the leaves on the depodded plant were still green six weeks later.

Mean weight and number of beans per plant, harvested at maturity, are shown in Table C1. Although partial depodding decreased the bean yield by 42%, the weight of 100 beans was the same in both podded and partially depodded plants.

Table C1. Mean bean yield and yield components ($\pm$ standard errors) for podded and partially depodded soybean plants on which F and Q were measured

	Bean weight per plant (g)	Bean number per plant	Weight of 100 beans (g)
Control (Podded)	154.3 $\pm$ 4.0	685 $\pm$ 7	22.5 $\pm$ 0.4
Partially depodded	89.3 $\pm$ 23.3	411 $\pm$ 129	22.1 $\pm$ 1.3

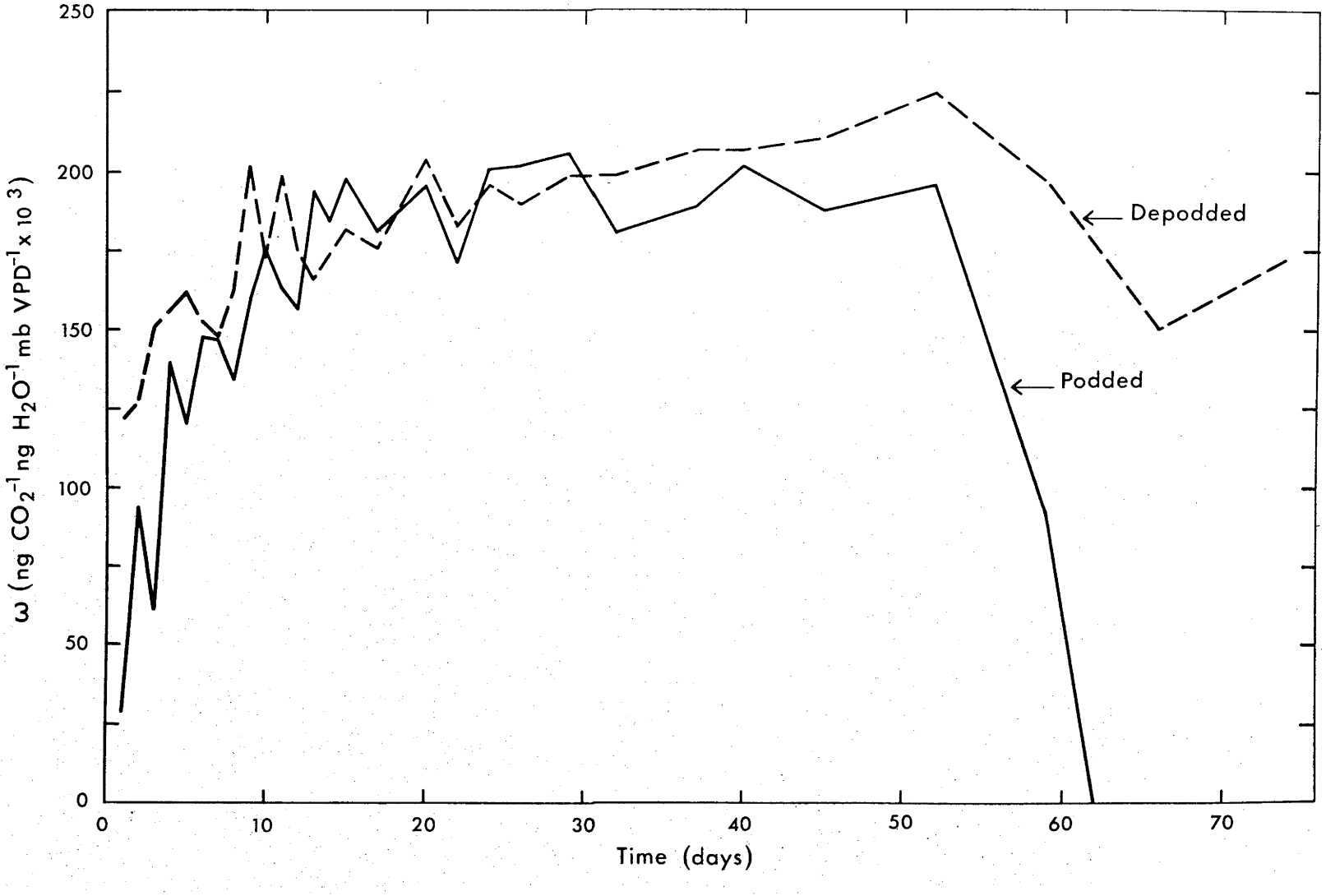

Fig. C6. Water use efficency (ω) for leaves on the main stem of partially depodded and untreated soybean plants from leaf emergence

DISCUSSION

Behaviour of F and Q with Time

Changes in F with time were similar to those in Q for the three sets of data (noted also for lucerne by Hodgkinson 1974) and so for convenience only F will be mentioned here. The pattern of F in soybean leaves was not the simple one observed in tobacco leaves where there was a steady increase in F as the leaf expanded up to 65-80% A_{max} followed by a marked and continual decline (Appendix II). In soybean, following the increase and peak in F associated with leaf expansion and the decline in F beyond the time of reaching A_{max} there was another increase and decline about the time of flowering and then further peaks during pod filling. Similar patterns of photosynthesis have been observed previously in both glasshouse (Section B) and field grown soybean plants (Zhailibaev and Khasenov 1966). It is possible that the peaks are inherent characteristics of leaves and their relation to flowering and pod filling are coincidental. This is discussed again later.

Behaviour of r_s and r_M with Time

Reflecting the similar behaviour of F and Q, r_s and r_M were also closely related over time in all sets of data, a finding similar to that of Kriedemann (1971) for orange leaves. Thus the water use efficiency (ω), a sensitive indicator of this relationship, was relatively stable between the time A_{max} was reached and the start of leaf senescence, when it declined to values observed in very young leaves. In tobacco, although r_s and r_M exhibited a similar relative parabolic decline and then increase as the leaf developed, they were not necessarily linked since both r_s and r_M equally caused the initial decline in F

after F_{max} was reached (Appendix II). No evidence of such a break in the r_s:r_M relationship appeared for leaves of soybean.

The Relative Importance of r_s and r_M in Limiting F and Possible Control Mechanisms

Photosynthesis has been plotted against stomatal and mesophyll conductances ($1/r_s$, $1/r_M$) for experiment 1 to determine the relationship between F and r_s and r_M (Fig. C7, and see Ludlow and Wilson 1971b for similar data on other dicotyledons). The stomatal and mesophyll conductances are highly correlated with F and both conductances are always partially limiting to F. However, the mesophyll (or residual) conductance was always the most limiting to CO_2 transfer. It has been proposed that the physical impedance to CO_2 diffusion through the mesophyll is important (Gaastra 1959; Brun and Cooper 1967; Kriedemann *et al.* 1970; Chartier *et al.* 1970; Jones and Slatyer 1972), but r_M also contains a biochemical component which may be equally important in limiting F (Wareing *et al.* 1968; Woolhouse 1968; Steer 1972; Bowes *et al.* 1972).

However, from this data I cannot say that the conductances control photosynthesis *per se*, although they do control the diffusion and carboxylation of CO_2 in the leaf. It is possible that these conductances are under the control of another plant factor, which may even be photosynthesis itself. Whether r_s and r_M were under the control of the same factor or whether r_s was being influenced by r_M is not clear. Other authors have shown that changes in r_M can influence r_s by altering the substomatal concentration of CO_2 and so affecting stomatal aperture (Meidner and Mansfield 1965) and that r_s can be changed by plant abscisic or phaseic acid concentrations (Jones and Mansfield 1970; Kriedemann

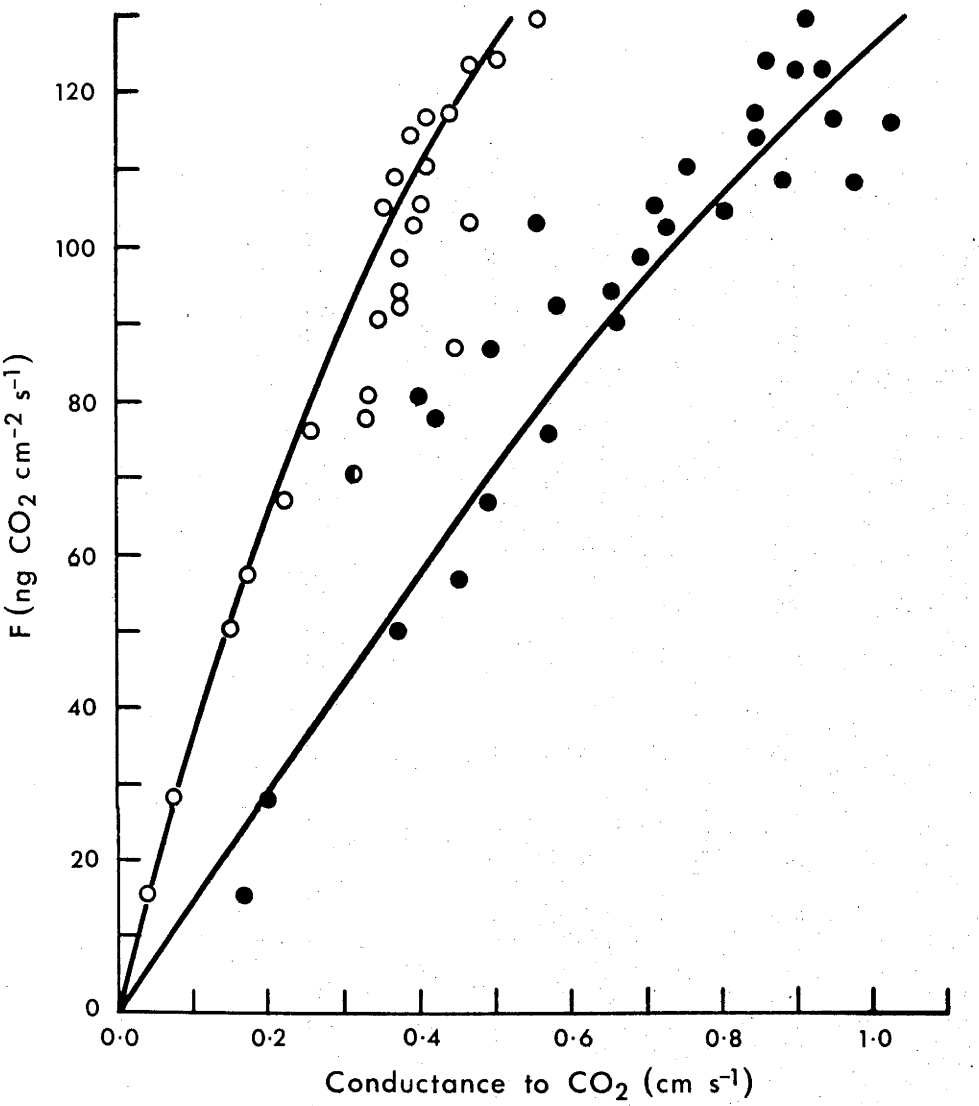

Fig. C7. Stomatal (●) and mesophyll (○) conductances plotted against net photosynthesis for leaves on the main stem of an untreated soybean plant (experiment 1)

et al. 1972). Thus the possibility remains that r_s and r_M can both be controlled by the plant and interact with each other. In accordance with this proposal, Upmeyer and Koller (1973) have shown from limited data that diurnal changes in F of soybean leaves held under constant conditions were associated with changes in both r_s and r_M and in leaf carbohydrate levels, and they suggested that a plant factor originating externally to the leaf was controlling the diffusive resistances.

Plant Control of F

It has been suggested that the troughs and peaks in the photosynthesis of soybean leaves are a result of a reproductive stimulus on F or are an inherent pattern in the leaf. The peaks do not appear to be associated with environmental variables, and if the cycling in F is controlled by the leaf or is an inherent pattern, I cannot explain why or how it occurs.

The association between an increase in F and flowering, and a trough and further peak in F during pod filling was observed in previous work (Section B) and there is further evidence to suggest that the behaviour of F is related to the reproductive behaviour of the plant. Increased net assimilation rate or increased photosynthesis has been measured during flowering and pod filling of soybean by Zhailibaev and Khasenov (1966), Koller *et al.* (1970), Dornhoff and Shibles (1970) and Ghorashy *et al.* (1971). Both flowering and pod filling are periods of rapid dry matter increase in soybean (Kumura 1969; Koller *et al.* 1970; Hardman and Brun 1971) and assimilate restriction during either of these stages reduces yield (Weber 1968; Hicks and Pendleton 1969). Therefore it is possible that the observed peaks in photosynthesis were related to

an increased requirement for assimilate. A parallel situation occurs in wheat where photosynthesis of the flag leaf may rise during rapid grain filling (Rawson and Evans 1971) and can be influenced by manipulation of the source and sink (King *et al.* 1967). However, it is surprising that the magnitudes and patterns of F and Q were almost identical in podded and partially depodded plants, particularly when there were pods in the axil of one leaf and there were no pods for six nodes below the other. The beans in the depodded situation did not benefit from the extra photosynthate available since the weight per seed was the same as in the podded plants. Assimilate surplus to the requirements of the beans in the depodded plants was presumably stored in the stems and leaves (cf. McAlister and Krober 1958). The greatest observable effect of pod removal was that photosynthesis was maintained for longer than normal, confirming an observation of Hicks and Pendleton (1969). Presumably mature pods encourage leaf senescence but this may be only a localised effect.

The results from the partial depodding experiment suggest that the factor resulting in the maintenance of F during pod filling is present not only in leaves adjacent to flowers or pods but also in the whole shoot system. Work by other authors indicates that changes in photosynthesis may involve not only the shoots but the whole plant. For example, an increased flux of cytokinins from the roots to the shoots occurs during flowering in *Perilla* and this has been associated with decreased leaf senescence (Beever and Woolhouse 1974). Similarly, Wareing *et al.* (1968) have shown that after partial defoliation photosynthesis of the remaining leaves increased under the stimulation of cytokinins from the roots and a subsequent increase in the activity of carboxydismutase.

In conclusion it seems that photosynthesis in the soybean plant may not be controlled by the reproductive sink size *per se* but may be influenced by growth substances from the roots or from reproductive tissues at critical growth stages. It is therefore somewhat restrictive to consider metabolic and physical changes in the leaf alone when attempting to explain the photosynthetic behaviour of single leaves, as I have done. It is important to also examine changes in the whole plant, especially the fluxes of growth substances known to influence F, when relating the changes in photosynthesis to plant development. Long-term measurements of F on vegetative plants may help elucidate some of the points raised here.

SECTION D: THE EFFECT OF ATMOSPHERIC HUMIDITY ON THE YIELD OF SOYBEAN

Abstract

Soybean plants (cv. Lee) were grown at high and low atmospheric humidities in controlled environments on two occasions. A constant head watering system ensured an adequate supply of water at all times. At low humidity the bean yields decreased as a result of a reduction in bean numbers which was only partly compensated by a small increase in bean weight. The level of humidity did not affect the protein or oil content of the beans. The lower humidity also reduced the dry weights of stems, leaves and total tops and the number of nodes per plant. A low humidity after flowering alone did not affect bean yield but did reduce the number of beans. It is suggested that the reduced bean yield at the lower humidity was the result of reduced assimilate production during the flowering and pod filling periods and mechanisms for this effect are discussed. The possible significance of the results to soybean production in the Australian environment is considered.

INTRODUCTION

Diffusive water loss from a leaf depends on the existence of a gradient of water vapour concentration between the substomatal cavities and the air outside the boundary layer. Air around the mesophyll cells is close to saturation under non-stress conditions, the actual concentration of water vapour depending on leaf temperature. Air outside the boundary layer of the leaf is generally not saturated, and since leaf temperature is usually within a degree or two of air temperature, there is a gradient of water vapour concentration. The larger this differential the greater the potential water loss from the leaf (Thut 1938; Whiteman and Koller 1967; Nevins and Loomis 1970; Aston 1973; Barrs 1973).

In the U.S.A. soybeans are grown primarily in the midwest where there are extensive areas of arable land with reliable summer rainfall and the atmospheric relative humidity is comparatively high (range 50-85% at summer temperatures, Anon. 1960). Such climates are rare in Australia except along the coastal fringe, and recent large expansion in soybean areas has occurred in inland districts where relative humidities are low during summer (range 30-60% at summer temperatures, Anon. 1960; Keig and McAlpine 1969). Hot, dry north-westerly winds are also common at this time (Gentilli 1972) and further increase potential transpiration from the crops by increasing the water vapour pressure differential and by reducing leaf boundary layers. Conditions likely to lead to plant water deficits are most likely to occur during January and February when the crops are at their most sensitive stages i.e. flowering and pod filling.

Low humidities and high transpiration rates may affect the yield of soybean in two ways. The first is associated with the "absorption lag" as a result of the high resistance to water transport in the root cortex of soybean (Kramer 1938; Boyer 1971). High potential transpiration rates exceed the rates at which the plants can absorb water from the soil, leaf water potential decreases and the stomata start to close, or if severe, the plant wilts (Boyer 1970 a,b; Aston 1973; Neumann *et al.* 1974). The second effect of low humidity is a direct partial closure of the stomata independent of the water potential of the leaf (Lange *et al.* 1971; Schulze *et al.* 1972). The effect of these responses on photosynthesis and yield has been discussed previously.

There have been comparatively few investigations into the effect of humidity on plant growth and economic yield (Sale 1970). Went (1957) concluded, after several experiments on diverse species, that atmospheric humidity had little effect on growth provided the water supply and the root system were adequate. Winneberger (1958) showed that very high relative humidity (approaching 100%) prevented the growth of pear buds, and reduced the growth rate of young sunflower plants. He concluded that transpiration was necessary for most higher land plants. Reduced dry matter production at very high relative humidity (approaching 100%) has also been demonstrated for cacao (Sale 1970), bean and cotton (Nieman and Poulsen 1967), and strawberries, broad bean and several root crops (Pareek *et al.* 1969). Very high humidities (at 30°C) have also caused abortion of all flowers on bean plants and severely retarded flower initiation in sunflower (Pareek *et al.* 1969), indicating hormonal linkages to transpiration or water deficits.

The depressed growth at very high relative humidity is no longer apparent when the relative humidity drops to about 90% or less. Increasing the daytime relative humidity from 45 to 90% increased the economic (dry) yield and total dry weight of beet and radish, and to a lesser degree of onion, in both saline and non-saline root media (Hoffman and Rawlins 1971). There was a linear relationship between leaf water potential (at 1100 h) and yield for the three root crops. Raising the relative humidity from 40% to 65% significantly increased the fresh weight, dry weight and leaf area in the horticultural species ageratum, petunia and marigold after 14 days (Krizek *et al.* 1971). Increasing the relative humidity from 65% to 90% did not significantly change the fresh or dry weights of these species. The seed yield and vegetative yield of sunflower was increased at higher humidities in both water and soil culture experiments conducted by Demidenko and Golle (1939). Ford and Thorne (1974), summarising nine years of research, found that an increase in atmospheric relative humidity (in the range 40% to 90%) increased the growth of sugar beet in all four experiments, the growth of kale in both experiments and the growth of wheat in three out of six experiments, although to varying degrees and not at all stages of growth.

The aim of this work was to determine the response of soybean to atmospheric humidity at different stages of growth. Soybean plants were grown to maturity in soil in large bins in two growth cabinets held continuously at high or low humidity, on two separate occasions. The plants were thus permitted to adapt to their environment. Plant and bean yields and their components were measured.

MATERIALS AND METHODS

Plant Culture for Both Experiments

Graded (220-240 mg), pre-germinated seed of soybean cv. Lee was sown in a fertile sandy-loam soil (CSIRO "Special Soil") in 0.05 m^3 bins. The seedlings were selectively thinned to one plant per pot during the expansion of the first trifoliolate leaf. A constant head subterranean watering system was introduced after the plants were established to supply unlimited water into 4 cm of coarse sand in the bottom of each bin. A complete nutrient solution was added to the top of the pots every fortnight.

Growth Conditions

The plants were grown in two controlled environment cabinets (*Controlled Environments Ltd.*, Winnipeg, Canada; Model PGW 36). Each cabinet held 8 bins with centres spaced 0.65 m apart. The light banks containing VHO CW fluorescent tubes and incandescent globes were raised as the plants grew so that the most recent leaves were always under a similar flux density. Irradiant flux density was about 65 J $cm^{-2}h^{-1}$ (total short-wave 400-1100 nm) or about 525 $\mu E\ m^{-2}s^{-1}$ (400-700 nm) near the top of the plants at the beginning of the second experiment.

Day and night air temperatures near the middle of the canopies were $30\pm0.5°C$ and $25\pm0.5°C$ respectively.

Air humidities were monitored by modified "Danfoss Hygrostats" (Martin *et al.* 1974) and their outputs were recorded every 30 min, converted to humidity values, and then averaged for each light and dark period. The sensors were calibrated against known vapour pressures over supersaturated salt solutions (Acheson 1965) and also by a water bath technique (see Appendix 1).

Experiment 1

Photoperiod was initially 15 h, with full irradiation for 14 h and day temperature for 14.5 h. Twenty-four days from seedling emergence, photoperiod was reduced to 12 h with 11 h of full irradiation and day temperature for 11.5 h.

Humidity control in the cabinets was only fair (Fig. D1). This was a function of the design of the conditioning system for air temperature which used a large heat exchanger and a cooling system with reverse-cycle heating. Reduced transpiration and increased dehumidification of the air during the dark period resulted in lower relative humidities than during the day. The system was also dependent on outside atmospheric humidity which was unusually high. The cabinets were modified to overcome these deficiencies before repeating the experiment.

Experiment 2

Initial photoperiod was 14 h and was reduced to 12 h 18 days after plant emergence. Day temperatures were held for 12 h during the high light period. The humidity treatments were exchanged between cabinets before the start of the second experiment. There was a greater humidity differential between treatments, higher relative humidities at night than during the day and more precise control of humidity in this experiment (Fig. D2).

At flowering (appearance of petals on several nodes) on day 36, four randomly chosen plants in each cabinet were moved to the other cabinet where they remained until maturity.

Leaf resistance (r_l, stomatal plus cuticular resistance) to water vapour diffusion was measured on a recently fully-expanded leaflet

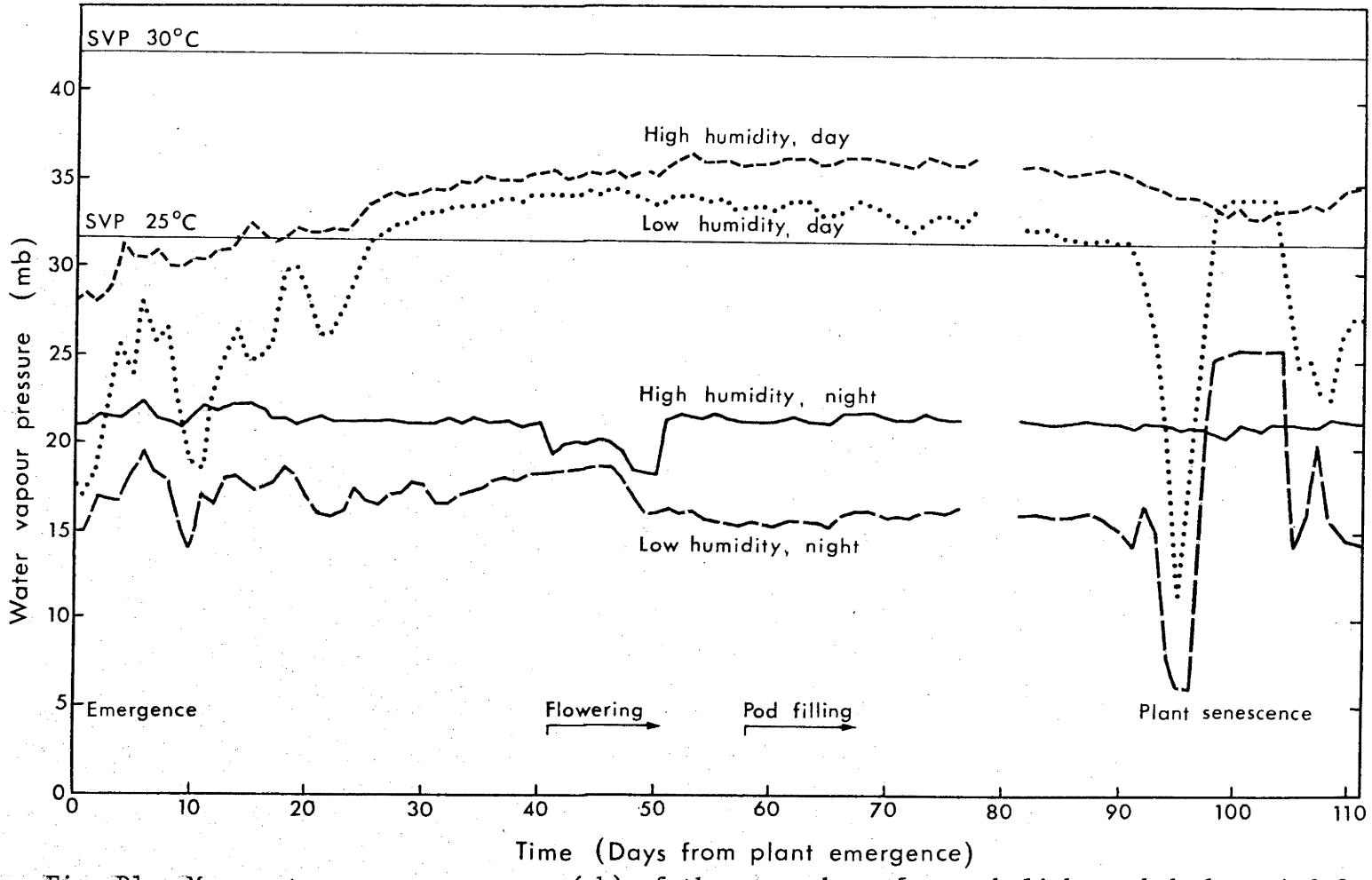

Fig. D1. Mean water vapour pressure (mb) of the atmosphere for each light and dark period for the low and high humidity growth cabinets during experiment 1, the saturated water vapour pressures at 30° and 25°C, and the times the plants reached various growth stages

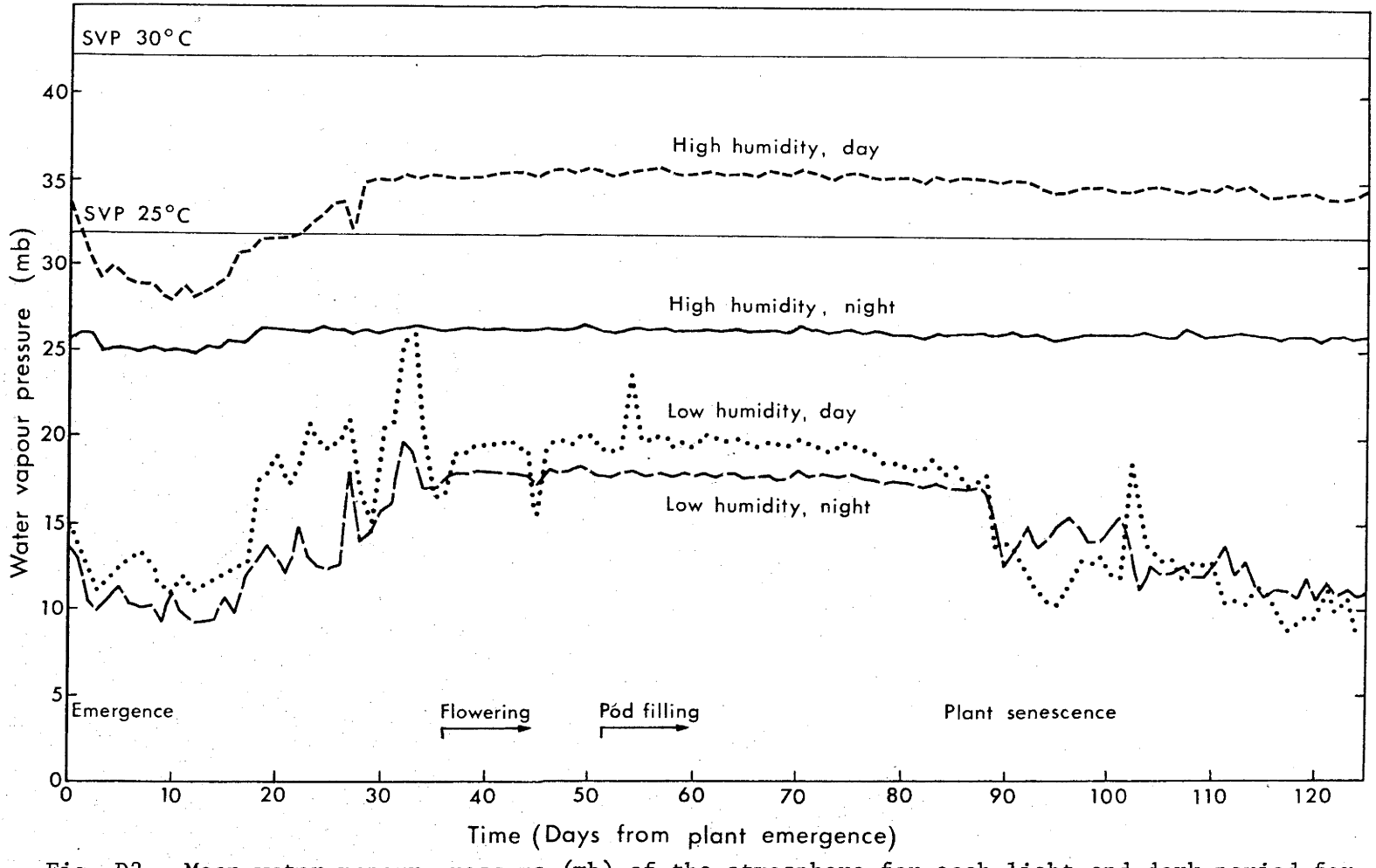

Fig. D2. Mean water vapour pressure (mb) of the atmosphere for each light and dark period for the low and high humidity growth cabinets during experiment 2, the saturated water vapour pressures at 30° and 25°C, and the times the plants reached various growth stages

on all plants during fowering and early pod filling with a self-timing aspirated diffusion porometer (Byrne *et al.* 1970).

Plant Harvests

Plants were harvested individually at maturity, except for the leaves which abscissed and so could only be collected for the whole cabinet. Leaves and stems were dried at 85°C for 24 h and weighed. Pods were threshed manually and weighed after drying at 35°C for 7 days. Pod number, seed number and seed weight (air dried) were recorded.

The oil content of the seed was determined by nuclear magnetic resonance (NMR); the protein content was determined by analysing for nitrogen by a modified Kjeldahl digestion (percent protein was equal to percent N x 6.25).

RESULTS

The times at which the plants reached different stages of growth are shown in Figs. D1 and D2.

Bean Yield and Its Components

The lower humidity reduced bean yield per plant by 12.3% in experiment 1 and 20.9% in experiment 2 (both differences significant at the 0.05 level of probability) - Table D1. Transferring plants from a high to a lower humidity at the start of flowering reduced yield by 12.7%, and transferring them from a low to a high humidity increased yield by 17.4%, but neither difference was significant. Humidity level prior to flowering had no significant effect on bean yield i.e. yield differences between the high and low/high treatments, and between the low and high/low treatments, were not significant.

Table D1. Bean yield and its components for soybean plants grown under different humidity levels in two experiments (with least significant differences)

Expt. No.	Humidity level	Bean yield per plant (g)	Bean number per plant	100 bean weight (g)	Pod number per plant
1	High	109.1	712	15.38	-
	Low	95.7	590	16.23	-
	LSD 0.05	11.0	70.9	1.29	
	0.01	15.3	98.8	1.80	
2	High	139.8	896	15.61	384
	Low	110.6	630	17.57	294
	High/low[A]	122.0	742	16.46	349
	Low/high	129.8	790	16.43	343
	LSD 0.05	20.2	114	1.30	50
	0.01	28.5	161	1.84	71

[A] Humidity level before and after flowering

The bean yeilds in experiment 2 were significantly higher than those in experiment 1, but the treatment by experiment interaction was not significant, indicating a similar effect of treatment on yield in both experiments

In order to determine the most sensitive stage of development the components of yeild must be examined.

Bean Number per Plant

The lower humidity reduced bean number per plant by 17.1% in experiment 1 and 29.7% in experiment 2 (both differences are significant at the 0.05 level of probability). The greater bean number at the higher humidity was a result of the production of more pods per plant, in particular, of significantly more pods with three beans.

Transferring the plants from a high to a lower humidity at the start of flowering caused a significant reduction (17.2%) in bean number, and transferring them from a low to a higher humidity caused a significant increase (25.4%) in bean number. Humidity level prior to flowering had no significant effect on bean number.

Mean bean number was significantly higher in experiment 2 than in experiment 1 (comparing only common treatments) and the treatment by experiment interaction was significant (at the 0.05 level of probability). Thus the residual mean square (within cabinet variation) may have underestimated the error term, but since the variance ratio was high for the treatment effect (34.60) this was not considered important. Also, improved humidity control in experiment 2 permitted a larger humidity differential between treatments, and the treatment x experiment interaction is seen as a consequence of this. Examination of the residuals showed that the variance was homogeneous, and so no transformation of the data was necessary.

Weight of 100 Beans

The 100 bean weight for the low humidity treatment was greater than that from the high humidity treatment although this was only significant in experiment 2. High humidity either before or after flowering

lowered the 100 bean weight, but the differences were not significant. This parameter is associated with the number of beans available for filling and total photosynthate production, and will be discussed later.

Chemical Composition of Beans

The protein and oil contents of the beans are shown in Table D2.

Table D2. Effect of humidity on chemical composition (expressed on dry weight) of beans from the two experiments

Experiment No.	Humidity level	Oil content, %	Protein content, %
1	High	24.22	43.86
	Low	24.72	43.61
2	High	23.14	44.91
	Low	21.98	45.18
	High/low	22.52	44.98
	Low/high	22.79	44.34

Oil Content

Humidity level had no significant effect on the oil content of the beans from experiment 1 or experiment 2. An analysis of covariance, with bean yield per plant as the covariate, also showed no significant effect of humidity on oil content, and no significant correlation between the parameters.

The moisture contents of the seed for NMR analysis were 2.1% and 2.6% in experiments 1 and 2 respectively. However, the measured oil content of the seed from the low humidity treatments (during pod filling) in experiment 2 continued to drop as the seed was dried to below 5% moisture content. This indicated that this seed contained less bound water than seed from the high humidity treatment. This free water apparently interferred with the NMR analysis. This may have important consequences on NMR methodology which until now has regarded 4-5% moisture as acceptable. It also means that water may have been incorporated into the seed in different ways in each humidity treatment.

Protein Content

Humidity had no significant effect on the protein content of the beans in either experiment and a covariance analysis with bean yield gave non-significant results.

Vegetative Plant Components

Leaf Dry Weight

The total dry weights of leaves from each treatment in experiment 1 were similar (Table D3). Obvious differences in plant vigour in experiment 2 prompted an attempt to harvest the leaves from each plant for analysis. The effect of humidity on leaf weight was not significant but this may be a result of harvest techniques rather than any real lack of differences.

Table D3. Effect of humidity on growth of soybean plants from two experiments (Plants were harvested at maturity; number of nodes refers to mainstem; least significant differences are presented.)

Expt. no.	Humidity level	Dry weight of: Stem (g per plant)	Leaf[A] (g per plant)	Total tops (g per plant)	Number of nodes per plant
1	High	77.4	77.8	311.6	21
	Low	67.9	77.1	274.7	21
	LSD 0.05	7.1			
	0.01	9.9			
2	High	81.5	67.0	355.4	18
	Low	49.2	57.7	264.8	16
	High/low	65.3	62.6	303.6	17
	Low/high	61.6	59.4	309.5	16
	LSD 0.05	7.0		(30.2)	2
	0.01	9.9		(42.7)	3

[A] Not analysed; mean values for each treatment available only.

Stem Dry Weight

High humidity significantly increased the stem dry weight by 14.0% in experiment 1 and by 65.7% in experiment 2, (both significant at the 0.05 level of probability). High humidity either before or after flowering significantly increased stem weight, compared to plants in

continuous low humidity. Low humidity at either stage of growth significantly reduced stem weight compared to plants in continuous high humidity. Thus significant dry matter accumulation in the stems occurred after flowering.

Total Dry Weight of Tops

The dry weight of plant tops was 13.4 and 34.2% higher at the high humidity in experiments 1 and 2 respectively compared to those at the lower humidity. A low humidity either before or after flowering decreased the total yield (by approximately 14%) compared to a continuously high humidity; whereas a high humidity at either stage of growth increased total yield (by approximately 16%), compared to a continuously low humidity. This parameter was not statistically analysed because it contains the leaf component which could not be estimated reliably for individual replicates.

Number of Nodes per Plant

The number of nodes on the main stem of each plant indicates the rate of development prior to flower initiation. In experiment 1 humidity had no significant effect on node number. In experiment 2 the plants kept in the lower humidity until flowering had significantly fewer nodes than plants from the high humidity. There were significantly fewer nodes on all plants from the second experiment as a result of the earlier reduction in photoperiod to initiate flowering.

DISCUSSION

When adequate supplies of water and nutrients were available, and disease was absent, the humidity of the atmosphere had a marked effect on the bean yield, and consequently the protein and oil yields, and on dry matter production of soybean plants. The principal component of the reduction in yield at the lower humidity was a reduction in pod number and thus bean number, which was not offset by the small increase in bean weight. The reduction in pod number was a result of floret abortion, rather than pod abortion or differences in the number of florets as a consequence of different plant sizes. Abortion of florets or young pods is regarded as a consequence of poor assimilate supply within the plant (Weber 1968; Hicks and Pendleton 1969; Hardman and Brun 1971). The relatively small increase in bean weight, despite reduced bean number, and the reduced growth rate and dry matter production at the lower humidity would indicate a reduced supply of assimilate at all growth stages, compared to plants at the high humidity. Even under favourable conditions, Hofstra (1972) has suggested that the growth of soybean would be limited by photosynthate supply at 30°C. Was the reduction in photosynthate a result of water stress at the lower humidity, or some other factor? This is now considered.

The values for leaf resistance (Fig. D3) to water vapour diffusion indicate that the degree of stress was such that the stomata did not close fully except possibly in one treatment where plants were transferred from the high to the lower humidity. (The leaves of these plants showed extreme variability in r_l, ranging from quite low values to those indicating closed stomata). Even so, r_l was higher in plants grown under the low humidity, and assuming no compensation in the meso-

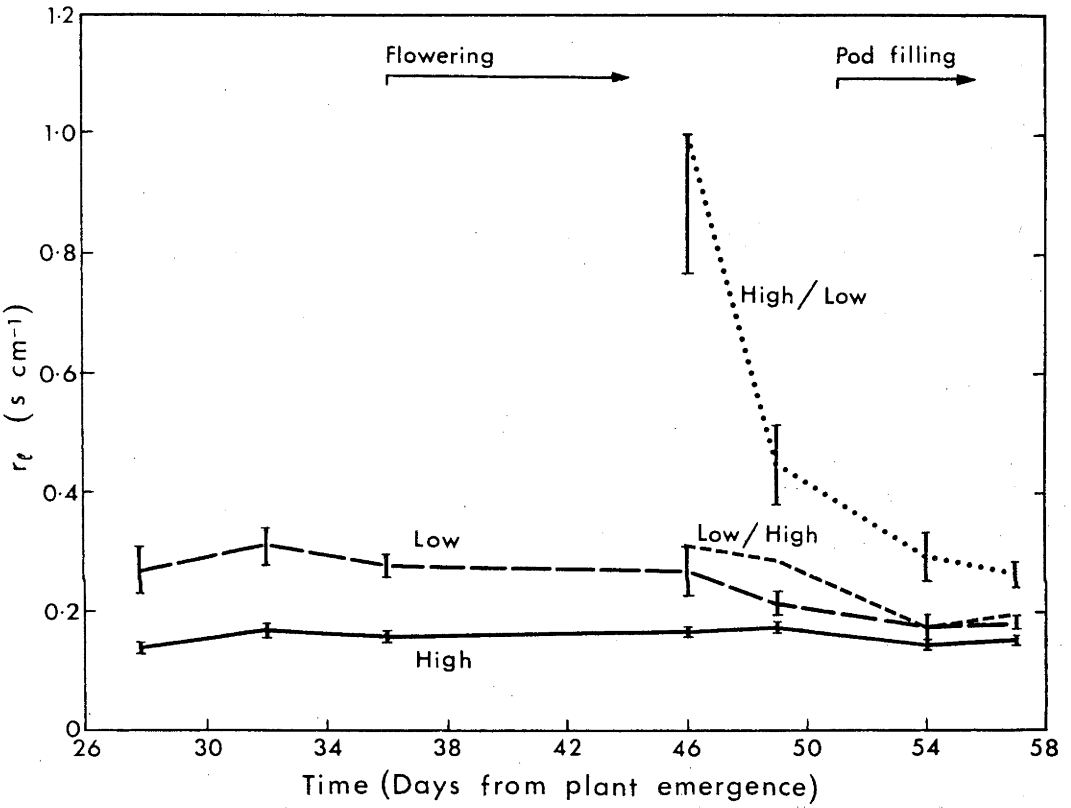

Fig. D3. Leaf resistance to water vapour diffusion (r_l) of recently fully-expanded leaflets during flowering and early pod filling for plants grown at different humidities (indicated on the figure); bars indicate $\pm$ standard error of each mean

phyll resistance to CO_2 transfer (cf. Section C) this would have resulted in reduced rates of photosynthesis (Boyer 1970b, Section C). This supports the hypothesis that reduced availability of assimilate caused floret abortion at the lower humidity. The higher r_1 values at the lower humidity may have been the result of lower leaf water potentials (compared to those at higher humidity), as a result of a rate of water loss from the leaf which exceeded the rate of supply (Boyer 1970b). The alternative hypothesis is that reduced photosynthesis was a result of stomatal response to humidity *per se* (Lange *et al.* 1971; Schulze *et al.* 1972), and the magnitude of the change in resistance supports this latter proposal (cf. Boyer 1970b; Schulze *et al.* 1972; Aston 1973). The absence of any effect of humidity on bean quality (oil and protein content of the seed) would also support this explanation, rather than one involving internal water stress which could affect biochemical pathways and quality parameters (Laing 1966 found that short stress periods significantly affected bean quality). Leaf water potential data would be required in order to state definitely which mechanism reduced photosynthesis in the plants, but this is not available.

The apparent adaptation of the plants to the low humidity, as shown by the decrease in leaf resistance at the end of flowering, may have been a result of hormonal changes associated with the reproductive phase (Meidner 1970; Beever and Woolhouse 1974) or may have been a true adaptive change. However, even this adaptation could not compensate for the previous loss of reproductive sites.

If the proposed explanation for reduction in yield at lower humidity is correct, it will prove difficult to overcome the direct effects of humidity on plant yield in the field in Australia. A broad-

acre spray irrigation technique to help increase humidity within the canopy would probably have only marginal benefit, as it would be impractical to irrigate during most of the day because of wind and high evaporation. Increased sowings in more humid areas near the coast could result in yield advantages, but other problems may arise. In intensive cropping areas of high humidity, disease and weed problems can adversely affect yields (Keogh 1974; Michael 1974) although their control with or without chemicals is feasible. Also many northern coastal areas of N.S.W. have relatively high autumn rainfall which would interfere with maturation of the crop and present harvesting problems.

In conclusion, consistent high yields of soybean in inland Australia may be difficult to obtain, because of the high evaporative demand and subsequent depression in photosynthesis, regardless of the water supply to the plant. Breeding or selecting soybean varieties with a lower root and stem resistance to water uptake and more resilient stomatal behaviour could help to increase yields in inland environments.

GENERAL DISCUSSION

The Relationship between Bean Quality and Yield

The small effect of the various treatments on the protein and oil content of the seed, despite some large differences in seed yield, was a consistent feature of the results. (Within experiments the greatest differences in the percentage of oil and protein were 1.4 and 1.9% respectively, while between experiments the range in mean oil and protein percentages was about 3%). Thus, protein and oil yields were proportional to bean yield. Bean quality may be influenced by the environment and cultivar (Laing 1974). Low temperatures and water deficits may reduce the oil content (Howell 1960; Laing 1966) while ineffective nodulation (and low availability of soil nitrogen) may reduce the protein content (cf. Laing 1974). It is surprising that bean quality was not affected by the water stresses in the first experiment (Section A), presumably neither the partitioning of assimilate within the seed nor oil synthesis were affected.

There is no genetic correlation between the protein content and bean yield of commercial soybean varieties, which are essentially dual purpose types (Hanson *et al.* 1961; Laing 1974). However, attempts to increase the protein yield of soybean by increasing the protein content (to over 50%) have been thwarted as the bean yields of these lines are lower (by 10 to 20%) than those of commercial varieties, although this may simply reflect a lack of breeding effort in this direction (Shibles *et al.* 1975). Generally, an increase in the protein content is associated with a reduction in the oil content and Hanson *et al.* (1961) estimated that in a breeding program for every 1% increase

in the protein percentage there would be a 1.5% drop in the oil percentage. Thus, at present, growing high protein varieties could be undesirable since one of the properties which gives the soybean producer some economic security would be lost and protein yields may not be significantly higher.

The most effective way of ensuring high yields of protein and oil with current varieties is by maximizing the bean yield. It could be desirable to increase protein and oil yields by breeding lines which have seed high in one of these respective characters, but attention shall also have to be given to maintaining or increasing the yield of these lines.

The Maintenance of Yield on Soybean

The components of yield in "determinant" soybean varieties demonstrate a high degree of plasticity. Compensation in one component can occur when another is adversely affected by the environment. Compensation for poor plant establishment can occur at a later stage of growth, such as in the number of flowers per plant. The pod number per plant and the bean number per pod are determined relatively early in the reproductive phase of the plant's life and thus most plasticity would be expected to occur in the bean weight parameter, which reflects environmental conditions during pod filling. However, plasticity from this source is unusual, as yields are often more highly correlated to the number of beans (or pods) per plant, or per ground unit area, than to the bean weight. This relationship has been observed over a range of environments, cultivars and treatments (e.g. Section D; Cooper and Brun 1967; Carter 1974; Laing 1974), so long as the bean filling period is

not stopped prematurely. Apparently it is ecologically advantageous for the plant to have a smaller number of beans fill adequately than to have a greater number only partially fill, or abort at various stages, as the result of lack of assimilate. Hardman and Brun (1971) have shown that increasing the number of pods set over that determined naturally, resulted in lower bean weights and no difference in yield (compared to untreated plants). This gives some idea of the plasticity of the plant.

It is proposed that the plant controls the number of beans it develops, according to environmental conditions, by a system of competition between the various growing points (or sinks) for the limited assimilate available, despite increased photosynthesis at critical stages (Section C). The early formed florets must compete with vegetative growing parts (cf. Sections A and D where such competition was demonstrated) and the nitrogen fixing nodules for photosynthate, and if the size of sinks determines their 'strength' and the distribution of assimilate as suggested by Evans (1975), the early formed floral tissue is at a disadvantage and may be nutritionally starved. Environmental conditions which are favourable to photosynthesis (e.g. high irradiation; air temperatures around 27°C; a good water supply) at this growth stage would reduce the number of pods that abort. It is not clear whether the vegetative parts or the reproductive tissue is the dominant sink during flowering as they both respond to increased availability of assimilate; although Shibles *et al.* (1975) interpreted the data of Hardman and Brun (1971) as indicating that the vegetative sink is markedly stronger (the results in Section A support this).

Later formed florets have to compete for assimilate with vegetative growth, root growth (Mitchell and Russell 1971) and also with

developing pods. These later florets would appear to be subordinate (at least partly) to those already developing and to the other sinks (Chen 1963). Unless conditions are favourable for photosynthesis many of these florets abort (Hardman and Brun 1971; Shibles *et al.* 1975). Since flowering and pod-set take place over several weeks (i.e. there is no critical stage) the soybean plant is thus apparently able to set the maximum number of pods that can probably be filled. If environmental conditions during the late pod filling period are more favourable (than earlier), yield compensation can take place through an increase in the bean weight component. However, adverse conditions during this period can severely affect bean yield through a reduction in the bean weight or the number of pods filled (Laing 1966). The limited supply of assimilate during flowering and pod filling would appear to be partly a result of relatively low leaf photosynthetic rates compared to other summer crops (El-Sharkawy and Hesketh 1965; Gifford 1974b) and the decline in photosynthesis after the leaf is fully expanded, although this latter factor may be moderated by the plant (cf. increases in photosynthesis after full leaf expansion in Sections B and C).

The Partitioning of Photosynthate

The partitioning of assimilate to late vegetative growth in the soybean would appear to be a barrier to the plant expressing its yield potential, at least in varieties in commercial use. Competition for assimilate between vegetative and reproductive parts during flowering and pod filling can be high, and excessive vegetative growth is not uncommon (Shibles and Weber 1966). Shibles and Weber (1967) obtained higher yields from treatments which promoted a high harvest index (in a

plant population study), i.e. in treatments that favoured partitioning of assimilate to the bean and not to vegetative tissue. Higher yielding varieties of wheat also partition a greater proportion of assimilate to the grain (Evans and Dunstone 1970). Blomquist and Kust (1971) classified translocation patterns in the soybean into only two categories related to apical dominance and later to pod filling; however, this may be an oversimplification as the movement of photosynthate within a soybean plant is quite flexible to the demands of specific sections of the plant (cf. Johnston and Pendleton 1968; Koller 1971).

There is little understanding of what determines the relative sink strengths or how they are related to translocation patterns in any species (Loomis *et al.* 1971; Evans 1975). Evans (1975) has suggested that the larger the sink the greater is its ability to compete for assimilate but a bias towards the storage organs is required at some stage and this bias can be influenced by the environment. It was suggested (Section A) that dominance for assimilate was related to the hormone balance of the whole plant, since a disruption to the roots appeared to provoke physiological changes in the tops. Indole-acetic acid and gibberellic acid have been shown to affect the rate and pattern of translocation in soybean seedlings (Hew *et al.* 1967), and an interaction between these two growth regulators and cytokinin may be responsible for apical dominance in *Phaseolus* (Field and Jackson 1974). An understanding of these processes may allow manipulation of relative sink strengths with consequent increases in yield; in the meantime, however, yield improvement must come from processes which are better understood. The concept of relative sink strengths and what determines or controls partitioning of assimilate is a major gap in our knowledge of plant physiology (Evans 1975).

Photosynthesis and Yield Improvement in the Soybean

Maximum photosynthetic rates in soybean leaves appear to be under endogenous plant control, within the physical constraints imposed upon CO_2 diffusion (Section C). The maximum rate would not appear to be influenced by sink size *per se* or leaf physiology *per se*; rather it appears to be under the influence of many partially limiting processes. This was apparent where a disruption to the primary (at that stage the reproductive) sink did not appear to affect photosynthesis; photosynthate was diverted to alternative storage sites in vegetative tissue (Section A and cf. depodding in Section C). The various peaks in photosynthesis during flowering and pod filling may have been the result of increased fluxes of growth regulators from the roots or developing reproductive tissue, as has been observed in other species (e.g. Beever and Woolhouse 1974; Loveys and Kriedemann 1974). Thus photosynthesis in soybean may be under hormonal control rather than a 'sink pull' mechanism of control. This does not exclude a negative feedback control system if photosynthate production exceeds demand (cf. Upmeyer and Koller 1973), although this may be rare in rapidly growing soybean plants.

Again it is interesting to note that high rates of photosynthesis were maintained in soybean leaves for long periods whereas leaf photosynthesis of tobacco, a "sink limited" plant, declined quickly after reaching a peak. This early decline may be the result of either a negative feedback mechanism or of the distribution of a growth regulator for which the younger leaves have a competitive advantage. Patterns of photosynthesis in these two species are well correlated with their respective growth habits.

Increasing photosynthesis within a soybean variety would probably increase yield. A similar conclusion was reached in a recent review by Shibles *et al.* (1975). This could be achieved by breeding plants within a variety for low CO_2 transfer resistances or with a different (hormone) control system. This would not be an easy task as these parameters are not easily determined, and large numbers of plants would have to be measured. An approach involving the application of 'growth substances' in order to influence photosynthesis or the partitioning of photosynthate, may produce results more quickly, but the method may be undesirable or impractical for field use. A yield increase from this source would be in addition to any derived by increasing photosynthate production by manipulation of the canopy e.g. by increasing the irradiance on the lower leaves (Shibles and Weber 1967; Johnston *et al.* 1969) or by improving the water environment of the canopy (Sections A and D).

A distinction should be made at this point between the advantages in breeding or selecting between varieties (or between species) for high rates of leaf photosynthesis, and breeding within a variety for high photosynthesis. Varieties or species with high leaf photosynthetic rates do not necessarily produce higher yields than those with lower rates (Curtis *et al.* 1969; Dornhoff and Shibles 1970; Gifford 1974b) because of different respiratory losses, differences in leaf area duration and in the duration of filling of the harvested organs, differences in the partitioning of assimilate, and differences in canopy architecture. Increasing the total photosynthate available during flowering and pod filling without affecting other plant parameters (such as leaf area or duration) should reduce competition for assimilate within a soybean plant and so permit more pods to form and subsequently fill.

There are many problems associated with breeding or selecting for plants with a high photosynthetic rate (Evans 1975; and cf. variation in photosynthesis described in Sections B and C). A rigorous set of standard conditions would be necessary for both the growth of the plants and the measurement of photosynthesis because of the many non-genetic causes of variation in photosynthetic rate (Ledig 1969). Many measurements of the same leaf would also have to be made. Even then unexplained variation may appear e.g. the maximum rate of photosynthesis of leaf 4 from plants described in Section C was substantially higher than those of leaves 3 and 5 described in Section B, and yet most of the apparently important environmental variables were accounted for (such as irradiance level, temperature, photoperiod, and nutrition). The factors influencing photosynthesis, especially those related to the physiology and environment of the roots, may not yet be understood well enough to permit plant selection on this basis.

Leaf and Plant Senescence

Leaf and plant senescence were retarded when pods did not fill properly or when pods were removed from a section of the plant. The leaves lost their dark green colour, appeared thick and had convoluted surfaces, and maintained low rates of photosynthesis until they died through lack of water. This maintenance of leaf activity would not normally be expected since a major sink for assimilate (the pods) was not present. The assimilate may have gone to the roots which could have continued to release hormones as a result of their stimulated growth. A localised hormonal balance in the depodded sections would appear to be involved as Hicks and Pendleton (1971) found that leaf senescence was

prevented only in sections of the plant where floral buds had been removed. Further, Beever and Woolhouse (1974) associated increased cytokinin flux from the roots, delayed leaf senescence and even regreening of yellow leaves, with disbudding in *Perilla*. Leaf senescence was hastened with the absence of floral induction and these authors suggested that cytokinin production was inhibited by vegetative growing points. It thus appears that the presence of reproductive growing points is a pre-requisite for these effects observed in soybean. This would appear to be further evidence suggesting hormones and their relative balance in the plant influence all major growth functions.

Concluding Comment

Increasing the quantity of assimilate available to developing flowers and pods would increase soybean yields. This could be accomplished at the level of the individual leaf by reducing the resistances to CO_2 transfer, reducing the sensitivity of the stomata to water deficits, by decreasing rates of photorespiration, or by encouraging maximum rates of photosynthesis for a longer period. It could be done at the level of the individual plant by altering the partitioning of assimilate between reproductive and vegetative structures and ensuring environmental factors which influence this relationship are favourable, or by changing the control system of photosynthesis, especially those 'growth substances' which appear to influence the carboxylation of CO_2. Increased photosynthate production could be achieved at the canopy level by increasing light penetration into dense canopies through narrow leaves and high leaf angles or by ensuring an adequate water supply. It could be done on a regional level by growing the crop in areas with the most

favourable environment e.g. areas with a long growing season and with high atmospheric humidities.

Elucidating and manipulating the major barriers to attaining high average yields of soybean is a challenge to agricultural scientists in many fields of research and extension. The effort is justified for it will help make Australia independent of vegetable oil and protein meal imports and could weld a strong link in the chain that will provide the people of less developed countries with an adequate diet for a healthy life.

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APPENDIX I. DESCRIPTION OF THE APPARATUS FOR MEASURING NET FLUXES OF CO_2 AND H_2O IN LEAVES AND METHODOLOGY FOR CALCULATING THE FLUXES AND DIFFUSIVE RESISTANCES IN THE LEAVES

Apparatus used for Photosynthetic Measurements reported in Sections A and B

An open system was used for measuring rates of photosynthesis of attached leaves; the air was conditioned for humidity and temperature before entering the assimilation chamber, and accurate control of leaf temperature and irradiant flux density was possible.

Conditioning of Air and Measurement of CO_2

Air was supplied by means of a large industrial compressor. The intake was situated well away from 'artificial' sources of CO_2, and some 4 km from the nearest residential area in the usual direction of wind flow. Consequently, CO_2 concentration during the measuring period exhibited a steady and predictable diurnal trend, falling from about 340 µl l^{-1} at 0900 h to about 320 µl l^{-1} at 1300 h. The air passed through an oil trap and particle filter, into a 0.2 m^3 mixing drum, then to the humidifier.

The humidifier consisted of two 3 l flasks, each containing 1.5 l of distilled water, submerged in a constant temperature ($\pm$ 0.1°C) water bath. The air percolated through four submerged porous blocks in each flask and exited through a tube at the top. The vapour pressure of water in the air stream was controlled by adjusting the temperature of the water bath. (The humidity of the air was checked using a $LiCl_2$ humidity sensor). The saturated air then entered a copper coil heat exchanger in a second controlled temperature bath in order to prevent the temperature of the air stream falling below its dewpoint in the air lines. The air entered the assimilation chamber above and below the leaf, and exited on the opposite wall. Float-type flowmeters (*Brooks Instrument Div.*, Pasadena, U.S.A.) calibrated against linear mass flowmeters (*Teledyne Hastings - Raydist*, Virginia, U.S.A.) monitored airflow in and

out of the chamber. Air samples (0.5 l min^{-1}) were taken before the first and after the second flowmeter for reference and sample air respectively. These were dehydrated by cooling to about 0°C before entering the infra-red gas analyser (IRGA). Air bleeds throughout the system ensured constant pressure. Air lines longer than 0.5 m were nylon pressure tubing instead of "Nylex" which was used for shorter distances.

A Grubb Parsons SB2 infra-red gas analyser (*Sir Howard Grubb Parsons & Co. Ltd.*, Newcastle upon Tyne, U.K.) with band pass filters to remove the water absorption wavelengths was used for the experiment described in Section A. A Uras 2 infra-red gas analyser (*Hartmann & Brown A.C.*, Frankfurt/Main, B.R.D.) was used for the work described in Section B. The CO_2 analysers were calibrated in the differential mode several times during each experiment using gases mixed with Wosthoff gas mixing pumps and a background CO_2 concentration of 300 µl l^{-1}. The millivolt output of the analyser was continuously recorded.

The Assimilation Chamber

The assimilation chamber was similar to that described by Jarvis and Slatyer (1966) and Jarvis *et al.* (1971), except that the small central chamber was not present. Briefly, it was constructed from "*Perspex*" and was 22 cm in diameter. Water jackets were around the top and bottom walls and all joints were sealed with neoprene "O" rings. The chamber possessed access ports for thermocouples and a photocell, and a fan in the bottom surface aided air movement. Leaf temperature was controlled primarily by the temperature of the water jackets. A resistance bulb thermometer situated just under the leaf was connected to a temperature-control unit which switched on heating or cooling coils in a water bath, depending on the temperature. Water was continuously recircul-

ated through this bath and through the jackets of the chamber. This gave excellent stability (± 0.1°C) and control of leaf temperature. Three 38 s.w.g. copper-constantan thermocouples were used to measure abaxial leaf surface and air temperatures. A silicon photocell (calibrated *in situ* against a *Lambda Instruments* (Lincoln, U.S.A.) quantum sensor, 400-700 nm) situated in the chamber near the leaf was used to measure irradiant flux density. The petiolule was sealed into the chamber using plasticine - damage was rare even after many measurements of the same leaflet.

Radiation Source

Sunlight was used to irradiate the leaves on glasshouse-grown plants described in Section A; this proved satisfactory because the translucent glass of the house acted as a diffuser. For later work (Section B) light from a 1500 W tungsten-halide lamp filtered through 5 cm of $CuSO_4$ solution to reduce the proportion of infra-red radiation was used. The flux density could be controlled up to 2200 μE m^{-2} s^{-1} by altering the distance between the lamp and the leaf.

Description of Apparatus and Methods where a Diffusion Resistance Analysis was Performed (Section C and Appendix II)

This equipment differed from that previously described because it was necessary to measure the water efflux from the leaf, the CO_2 concentration of the ambient air and CO_2 compensation points.

Conditioning of Air and Measurement of CO_2 and H_2O Vapour

Air was drawn by a double diaphragm pump from outside the building, through a particle filter and over a cooling coil held at 2°C for dehumidification. The air was rehumidified by passing it through a

heated chamber into which water was pumped (Milton Roy C minipump) at an accurately controlled and predetermined rate. The air then passed to a 0.2 m^3 mixing drum. Three air samples were drawn off after the drum; the first was dehumidifed by cooling to about 0°C and passed through a URAS 2 IRGA for measurement of CO_2 concentration. This IRGA was calibrated in the absolute mode (0-400 $\mu l\ l^{-1}$) and high purity dry N_2 was used as the reference gas. The second sample was passed through one side of a differential psychrometer (Bierhuizen and Slatyer 1964) which was calibrated by adding a known amount of water to an air stream, using the Milton-Roy mini pump and heated chamber; a theoretical calibration based on psychrometry theory was used only as a check and agreed well. The third sample provided a reference for one side of the differential IRGA. The main air line fed two assimilation chambers, with appropriately placed flowmeters for measurement of flow rate and as a check for chamber leaks. The outgoing air was sampled twice, one sample for the second side of the differential psychrometer, and the other, after drying, for the sample cell of a URAS 2 IRGA (for experiments described in Appendix II) or an A.D.C. IRGA (*Analytical Development Co. Ltd.*, London, U.K.) for measurement of the CO_2 differential across the chamber.

The differential psychrometer was positioned in a water bath held at 27.2 $\pm$ 0.1°C, and measured the wet bulb temperature of the air entering the assimilation chamber and the increase in wet bulb temperature from the outgoing air. Output voltages were continuously measured on a chart recorder.

The Assimilation Chambers

The "*Perspex*" assimilation chambers measured 22 x 17 x 2 cm and had water jackets around the top and bottom surfaces. The temperature of

the water in the jackets was controlled (± 0.1°C) and the water contained $CuSO_4$ for absorption of infra-red radiation. Air velocity through the chamber was increased by a diaphragm pump which recirculated air through the chamber at 35 l min^{-1} to ensure a low boundary layer resistance. Air sealing was accomplished with foam rubber (Sellotape 2600). Thermocouples (38 wire-gauge Cu/Co) were used to measure air and leaf temperatures.

Radiation Source

Two 400 W HLRG mercury vapour lamps and one 300 W tungsten spotlamp irradiated each chamber. These provided an irradiant flux density of between 1000 and 1900 μE m^{-2} s^{-1} on the adaxial leaf surface depending on the distance from the light source to the leaf.

CO_2 Compensation Point

CO_2 compensation points of leaves were measured in a closed system. A low capacity peristaltic pump recirculated air slowly through a leaf chamber (12 x 8 x 2 cm), into a dehumidifier (≈0°C), into the URAS 2 (also used for absolute measurement of CO_2 in the reference air) and back to the chamber. The pump was switched on intermittently to minimise the chance of leaks, and the minimum CO_2 concentration recorded in the system was taken as the CO_2 compensation point for calculation of total leaf resistance to CO_2 diffusion.

Calculation of Photosynthesis, Transpiration and Resistance to CO_2 Diffusion in the Leaf

A programmable desk calculator (Hewlett Packard 9820A) was used to calculate photosynthetic rates, transpiration rates and the resistances to these fluxes in the leaf. Resistances were calculated according to the

method first outlined by Gaastra (1959) and discussed by Jarvis (1971). A few points of clarification are required. Total resistance to CO_2 diffusion from outside the leaf to the reaction site in the chloroplast (r_{CO_2}) was calculated by

$$r_{CO_2} = (C_a - C_c) / F$$

where C_a is the CO_2 concentration in the external air (ng cm^{-3}), C_c is the CO_2 concentration at the carboxylation site taken as the CO_2 compensation point (ng cm^{-3}), and F is the flux of CO_2 into the leaf (ng cm^{-2} s^{-1}). C_a was determined by measuring the CO_2 concentration in the recirculating air system and was found to be the same as the air leaving the chamber. The boundary layer resistance (r_a) was determined by first measuring water efflux from water saturated "*Wettex*" of different sizes cut to the shape of the leaves being measured. This was better than the traditional filter paper method because of the superior water holding capacity and surface texture of the "*Wettex*". This value was then converted to a resistance to CO_2 by

$$(r_a)_{CO_2} = (r_a)_{H_2O} \cdot (D_{H_2O}/D_{CO_2})^{2/3}$$

(D_{H_2O}/D_{CO_2}) was taken as 1.605 (Fuller *et al.* 1966). The stomatal resistance (r_s) was determined by measuring the water efflux from the leaf and then deriving the resistance to CO_2 by

$$(r_s)_{CO_2} = (r_a + r_s)_{H_2O} \cdot (D_{H_2O}/D_{CO_2}) - (r_a)_{CO_2}$$

This assumes a high cuticular resistance which is not calculated. For the

calculation of $(r_a + r_s)_{H_2O}$, the water vapour concentration around the leaf was assumed equal to that in the recirculating system which, averaged over many comparisons, was found to be that of the in-going air plus 0.7 times the differential across the chamber.

The residual or mesophyll resistance (r_M) was then derived by difference -

$$r_M = r_{CO_2} - (r_a)_{CO_2} - (r_s)_{CO_2}$$

The program is presented on the following pages.

```
0:
ENT "AREA CM2",R
4⊢
1:
ENT "TEMP. LC AI
R 'C",R6⊢
2:
ENT "TRANSP WETT
EX",R1;ENT "FLOW
 L/MIN",R14⊢
3:
(R1-.6167)/.5615
→R11⊢
4:
R11*10↑4*R14/(6*
R4)→R33⊢
```

Area of "Wettex leaf", temperature of air, flowrate and differential psychrometer output are entered

Transpiration rate, Q, of Wettex, ng H_2O $cm^{-2}s^{-1}$

```
5:
ENT "TEMP WET BU
LB 'C",R12⊢
6:
ENT "TEMP DRY BU
LB",R9⊢
7:
R1/10.54→R2⊢
8:
R12+R2*0.7→R5⊢
9:
6.1078EXP (17.26
939R5/(237.3+R5)
)-.66(R9-R5)(1+1
.1E-3R5)→R34⊢
10:
R34*217*10↑3/(27
3.16+R6)→R35⊢
```

Calculation of concentration of water vapour in air surrounding the Wettex

ng cm^{-3}

```
11:
ENT "TEMP WETTEX
 'C",R13⊢
12:
2.17E5*6.1078
EXP (17.26939R13
/(237.3+R13))/(2
73.16+R13)→R36⊢
```

Calculation of concentration of water vapour on surface of Wettex

ng cm^{-3}

```
13:
(R36-R35)/R33→R3
7;R37*1.605↑(2/3
)→R38;PRT "(RA)C
02",R38⊢
14:
PRT "-----------
-----"⊢
15:
GTO 0⊢
16:
END ⊢
```

Calculation of $r_{a(H_2O)}$ which is then converted to $r_{a(CO_2)}$, s cm^{-1}

Calculation of leaf F, Q, r_a, r_s, r_M, and ratios of these quantities

0: ENT "DELTA CO2 P PM",R1;FXD 5⊢ 1: ENT "FLOWRATE L/ MIN",R2⊢ 2: ENT "TEMP. LEAF 'C",R3⊢ 3: ENT "LEAF AREA C M2",R4⊢ 4: R1*44.01*273.16* R2*100/(6*R4*22. 414(273.16+R3))→ R20⊢ 5: PRT R20,"NG CO2/ CM2/SEC"⊢	ΔCO_2, air flowrate, leaf temperature and area are entered for calculation of F F, ng CO_2 $cm^{-2}s^{-1}$
6: ENT "AIR CO2 PPM ",R5⊢ 7: ENT "TEMP. LC AI R 'C",R6⊢ 8: R5*44.01*273.16/ 22.414(273.16+R6)→R21⊢ 9: ENT "CO2 COMP PO INT",R7⊢ 10: R7*44.01*273.16/ 22.414(273.16+R3)→R22⊢ 11: (R21-R22)/R20→R2 3⊢	Calculation of total resistance to CO_2 from ... Concentration of CO_2 in air, ng cm^{-3} Concentration of CO_2 in chloroplast, ng cm^{-3}. r_{CO_2}, s cm^{-1}
12: ENT "DIFF. PSYCH CHAR",R50;(R50- .6167)/.5615→R8⊢ 13: R8*10↑4*R2/(6*R4)→R24⊢ 14: PRT R24,"NG H20/ CM2/SEC";SPC 1⊢	Calculation of water efflux of leaf from output of differential psychrometer, flowrate and leaf area Q, ng H_2O $cm^{-2}s^{-1}$
15:	

continued

```
15:
PRT R23,"TOTAL R
ESISTANCE"⊢
16:
ENT "TEMP DRY BU
LB 'C",R9⊢
17:
ENT "TEMP WET BU
LB 'C",R10⊢
18:
R50/10.54→R51⊢
19:
R10+R51*0.7→R53⊢
20:
6.1078EXP (17.26
939R53/(237.3+R5
3))-.66(R9-R53)(
1+1.15E-3R53)→R2
5⊢
21:
R25*217*10↑3/(27
3.16+R6)→R26⊢
22:
6.1078EXP (17.26
939R3/(237.3+R3)
)→R60;2.17E5*R60
/(273.16+R3)→R27
⊢
23:
(R27-R26)/R24→R2
8;R28*1.605→R29⊢
24:
PRT R29,"RA+RS R
ESISTANCE";SPC 1
⊢
```

Calculation of total resistance to water efflux ($r_a + r_s$) from ...

Concentration of water vapour in air around the leaf, ng cm^{-3}

Concentration of water vapour in substomatal cavity, ng cm^{-3} then conversion to resistance to CO_2 diffusion, s cm^{-1}

```
25:
0.15613LN (1+R4)
→R38;PRT "(RA)CO
2",R38⊢
```

Calculation of $r_{a(CO_2)}$ from function derived elsewhere, s cm^{-1}

```
26:
R29-R38→R31⊢
27:
PRT "(RS)CO2",R3
1⊢
```

Calculation of $r_{s(CO_2)}$ by difference, s cm^{-1}

```
28:
R23-R38-R31→R32⊢
29:
PRT "(RM)CO2",R3
2⊢
30:
```

Calculation of $r_{M(CO_2)}$ by difference, s cm^{-1}

continued

```
30:
R32/R31→R62;SPC
1;PRT "RM:RS",R6
2⊢
31:
R20/(R24/(R60-R2
5))→R61;SPC 1;
PRT "NG CO2/NG H
20/MB",R61⊢
32:
PRT "NG H20/MB D
EFICT",R24/(R60-
R25)⊢
33:
PRT "-----------
-----"⊢
34:
GTO 0⊢
35:
END ⊢
```

Calculation of ratios-

$r_M : r_s$

F:Q mb VPD^{-1}

Q mb VPD^{-1}

Example of output for one set of measurements

```
        103.50778
NG CO2/CM2/SEC
       7098.27572
NG H20/CM2/SEC

          4.60588
TOTAL RESISTANCE
          2.11901
RA+RS RESISTANCE

(RA)CO2
           .68456
(RS)CO2
          1.43446
(RM)CO2
          2.48687

RM:RS
          1.73367

NG CO2/NG H20/MB
           .19071
NG H20/MB DEFICT
        542.74545
----------------
```

APPENDIX II. PHOTOSYNTHESIS AND TRANSPIRATION OF EXPANDING LEAVES OF TOBACCO AND SUNFLOWER

Abstract

The aims of this investigation were (1) to determine if irradiance and nutrition can influence the timing of peak photosynthesis (F_{max}) in expanding tobacco leaves and (2) to assess the relative importance of stomatal (r_s) and mesophyll (r_M) resistances to CO_2 transfer as leaves expand.

Growth conditions affected rates of leaf expansion and final leaf areas (A_{max}), but not the time from leaf emergence to A_{max} (25 days). Patterns of photosynthesis with time were similar in all treatments; F rose rapidly to F_{max} and then declined; F_{max} occurred on day 13 when the areas of leaves in different treatments were between 65 and 80% A_{max}. It is suggested that temperature may determine the timing of F_{max}.

Changes in both r_s and r_M were associated with changes in F. Although changes in r_M were up to five times greater than in r_s prior to F_{max}, the relative reduction in the two resistances was similar. Absolute changes in r_s and r_M were similar immediately after F_{max} and during this period of seven days F declined by almost 50%. Thereafter, relative changes were again similar. Possible mechanisms for the control of the resistances by the plant are discussed. The hypothesis, that F_{max} was maintained for only a limited period in expanding tobacco leaves because of the lack of a sink for assimilate, is discussed. Expanding sunflower leaves maintained F_{max} for a longer period and reasons are presented for this different behaviour.

The water use efficiency (ω) of tobacco leaves changed as they expanded and was highest under good nutrition and lowest under low irradiance. Maximum ω for tobacco and sunflower leaves was similar.

INTRODUCTION

Leaves of tobacco plants grown under adequate nutrition and summer irradiation reached maximum rates of net photosynthesis per unit area (F_{max}) very early in their expansion (around 40% of final area, 40% A_{max}). With further expansion F declined rapidly so that by the time A_{max} was reached it was as low as 30% F_{max} (Rawson and Hackett 1974). However, F does not always decline early in tobacco as can be shown from Sestak and Catsky (1961), Wada (1968) and Wada and Kuroda (1968). A rapid decline in F of fully expanded leaves is not uncommon in dicotyledons (e.g. Woolhouse 1968; Ludlow and Wilson 1971b) but there are few examples of species which show a fall early in leaf expansion, except where poor nutrition is the cause (Natr 1972).

An aim of this work, therefore, was to examine whether the timing of F_{max} in relation to A_{max} can be influenced by irradiance level and nutrition, in a single tobacco variety. It appears that either stomatal resistance (r_s) or mesophyll resistance (r_M) may predominate in controlling CO_2 transfer in leaves of different species (Ludlow and Wilson 1971b). By an analysis of diffusive resistances, it was aimed to determine the dominant resistance associated with the decline in F after F_{max} in tobacco. Once the factors associated with the photosynthesis patterns of expanding leaves are known, we may be able to manipulate them to our advantage. Some results are also presented for sunflower. These experiments provide additional information which helps to elucidate the reasons for the patterns of photosynthesis relative to plant development in soybean.

MATERIALS AND METHODS

Plant Culture

Tobacco plants (*Nicotiana tabacum L.* cv. Mammoth 17L) were grown in 16 cm pots filled with a mixture of river sand, peat and loam and were exposed to an irradiant flux density of 480 μE m^{-2} s^{-1} (400-700 nm) provided by cool white fluorescent and incandescent lamps for 12 h each day. (Irradiance measurements were made with a Lambda Instruments quantum sensor). Day and night temperatures were 27°C and 22°C respectively. After the twelfth leaf emerged a complete nutrient solution was added to the pots twice each week in addition to daily watering.

Two similar populations, each of six plants, were selected when the twelfth leaf was approximately 1 cm^2. One population was exposed to light of 780 μE m^{-2} s^{-1}, hereafter known as high light or HL, and the other population was exposed to 260 μE m^{-2} s^{-1} (low light or LL) by adjusting the distance from the lamps to the plants. Temperature and daylength were not changed. Final leaf numbers were greater than 50 per plant.

A third population of plants which received a heavy basal dose of nutrients was raised under similar conditions up to the appearance of leaf 10. Thereafter a complete nutrient solution was supplied every two days. When leaf 12 emerged the irradiance level was adjusted to 480 μE m^{-2} s^{-1}, intermediate between HL and LL. This treatment will be referred to as high nutrition (HN).

The sunflower plants (*Helianthus annuus L.* cv. Peredovik) were grown in a glasshouse during winter in 25 cm pots filled with a mixture of perlite and vermiculite; water and nutrients were supplied daily. The glasshouse temperature was 27°C during the day and 22°C at night. When the leaf to be measured emerged (leaf 14), the plants were moved permanently to

a growth cabinet providing an irradiance level of 1075 μE m^{-2} s^{-1}. Mature plants had 25 leaves.

Gas Exchange Measurements

The gas exchange of leaf 12 was measured between 10% A_{max} and 100% A_{max} on five or six plants from each of the three treatments. Up to six measurements were made on each tobacco leaf.

Photosynthesis measurements were made with a URAS II infra-red gas analyser and leaf transpiration was measured with a differential psychrometer. A detailed description of the apparatus is given in Appendix I. Air temperature in the chamber was maintained at 25.4 ± 0.1°C, and leaf temperature averaged over the experiments was 27.7 ± 0.1°C; temperatures were higher for older leaves. The carbon dioxide concentration of air entering the chamber was 340 μl l^{-1} at the commencement of the experiment (August 1974) and fell progressively to 324 μl l^{-1} (October 1974). Air flow rates were adjusted so that carbon dioxide levels were usually above 300 μl l^{-1} and never below 290 μl l^{-1}. Wind speed through the chamber was about 1.5 m min^{-1}, achieved by recirculating air at 35 l min^{-1} mixed with fresh air at up to 20 l min^{-1}. The vapour pressure deficit of fresh air entering the chamber was controlled at 14.6 mb.

Calculation of Diffusive Resistances

Calculation of the leaf resistances to CO_2 diffusion followed Gaastra (1959) and is described in detail in Appendix I. The vapour pressure deficit of air around the leaf was estimated from that of the recirculated air. r_M was as defined by Ludlow and Wilson (1971a). Carbon dioxide compensation points of leaves were estimated by changing from an open to a closed recirculating gas system. To ensure that leaf temperature was unaltered by this change (cf. Williams and Markley 1973) the incand-

escent lamp was turned off which reduced the irradiant flux density on the leaf to 1170 μE m^{-2} s^{-1}. This was considered to be sufficient for leaves which were grown at 780 μE m^{-2} s^{-1} (cf. Jackson and Volk 1970). A measurement of CO_2 compensation point took from 1-2 h, while a determination of F took from 3-4 h before stability. The CO_2 compensation points for tobacco leaves fell progressively with leaf expansion from about 85 μl l^{-1} at day 1 to 45 μl l^{-1} at day 30. For high nutrition plants the fall was much steeper, being 35 μl l^{-1} by day 12 and remaining there until day 25.

Stomatal resistances (r_s) to water vapour diffusion for sunflower leaves were estimated with an aspirated diffusion porometer (Byrne *et al.* 1970). F was measured as described elsewhere except that a small leaf chamber was used (3 x 2 x 2 cm) and both sides of the leaves were examined independently. The chamber was originally positioned in the centre of a very young leaf, 2-3 cm wide, and on subsequent occasions it was positioned within the distal portion of this same area.

Leaf areas were measured on intact leaves from all treatments at least three times each week with Studio Proof Paper F and an electronic planimeter. A Richards curve (1959) was fitted to each leaf to describe its expansion. From these fitted curves, the commencement of leaf expansion (day 0) was estimated, and the gas analysis data for individual leaves were referred to this day.

RESULTS

The results for tobacco are presented first and are followed by a comparison with sunflower.

Photosynthesis and Leaf Expansion

Leaves expanded faster and were larger when grown under low light or with high nutrition. (HN leaves expanded under irradiance intermediate between HL and LL and, in the absence of improved nutrition, would probably have had intermediate growth characteristics). However, patterns in F prior to the attainment of A_{max} and the times taken to reach A_{max} were similar in all treatments (Fig. AP1). In all treatments the rise in F was rapid and reached a peak at approximately day 13, and was followed by a rapid decline to about day 23. F of low light leaves was then maintained at a low rate but F of high light leaves declined.

Although the timing of F_{max} was similar in the three treatments, leaves were at different stages of expansion ranging between 65 and 80% A_{max}. Rawson and Hackett (1974; shown as dotted lines in Fig. AP1) found that F_{max} occurred at 40% A_{max} on day 13.

Water Flux from Expanding Leaves

Net mass flux of water vapour exchange per unit leaf area (Q), expressed per millibar vapour pressure deficit, is presented in Fig. AP2. The peak Q of about 550 ng H_2O cm^{-2} s^{-1} mb^{-1} coincided with F_{max}. At this stage Q was twice that at day 5, while during the same period F had increased four-fold. Beyond about day 13 there was a progressive decline in water loss. The decline was more rapid in leaves grown with better nutrition so that by A_{max} these leaves were losing approximately 50% less water. On the other hand, low irradiance levels apparently kept water loss high.

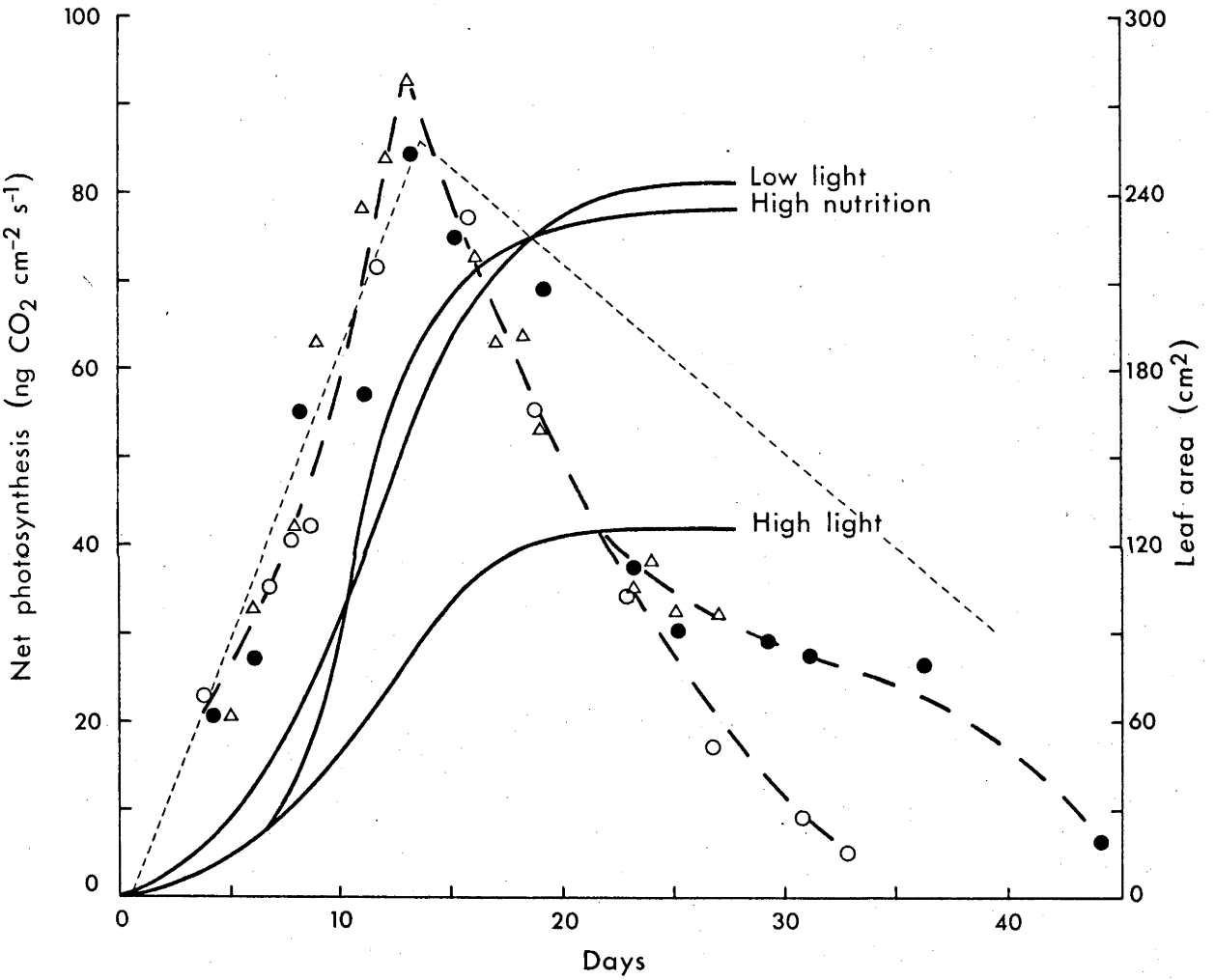

Fig. AP1. Leaf areas (solid lines) and net photosynthesis (dashed lines) for tobacco leaves expanding under high light (○), low light (●) and intermediate light and high nutrition (△). The photosynthesis curve from Rawson and Hackett (1974) is shown (dotted line)

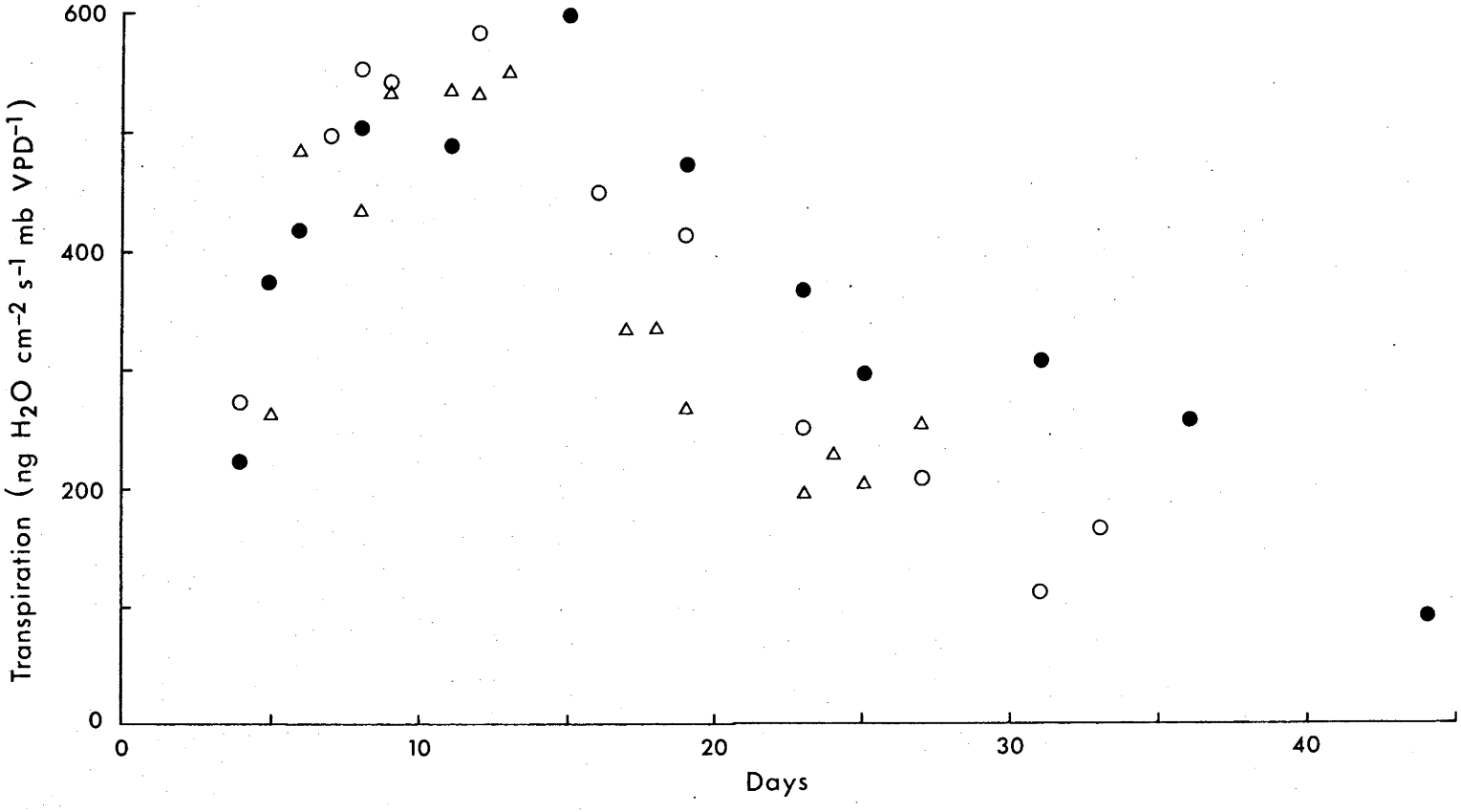

Fig. AP2. Water use by tobacco leaves expanding under high light (○), low light (●) and intermediate light with high nutrition (△)

Changes in Resistance to CO_2 Diffusion

Boundary layer resistance (r_a) increased linearly as leaf area increased from 10 to 120 cm^2, but above 120 cm^2, r_a scarcely increased. For example, r_a values for leaves of 10 cm^2, 120 cm^2 and 200 cm^2 were 0.5, 1.0 and 1.15 s cm^{-1}. From this information and Fig. AP1, r_a values at different stages of leaf development can be estimated.

Residual or mesophyll resistances (r_M) were three to five times greater than r_s (cf. ordinate on Fig. AP3). Before day 13 (F_{max}) and between days 20 and 25 (A_{max}) relative changes in the two resistances appeared to be similar in all treatments. Between days 13 and 20 there was a greater relative increase in r_s than in r_M for leaves grown with high nutrition or high light, but absolute changes in these resistances were similar.

Changes in Water Use Efficiency

The efficiency of water use (ω) is defined as the mass of carbon dioxide fixed for each unit mass of water transpired (i.e. F/Q), and to facilitate comparison with other experiments, the data are expressed per millibar vapour pressure deficit (mb VPD). By definition ω is a sensitive indicator of the relationship between r_s and r_M and so can be used to support data in Fig. AP3. The ratio of r_M:r_s is less satisfactory as any errors arising in calculating the resistances are accentuated (r_M is derived by difference between the total resistance and the sum of r_a and r_s and therefore any errors in r_s are reflected in r_M). Thus in expanding leaves up to about day 13, progressively more CO_2 was being fixed per unit of water used, thus r_M must have been declining more rapidly than r_s (Fig. AP4; in Fig. AP3, the resistances appeared to be linked during this period). Similarly, after about day 20 (80-90% A_{max}), ω declined rapidly

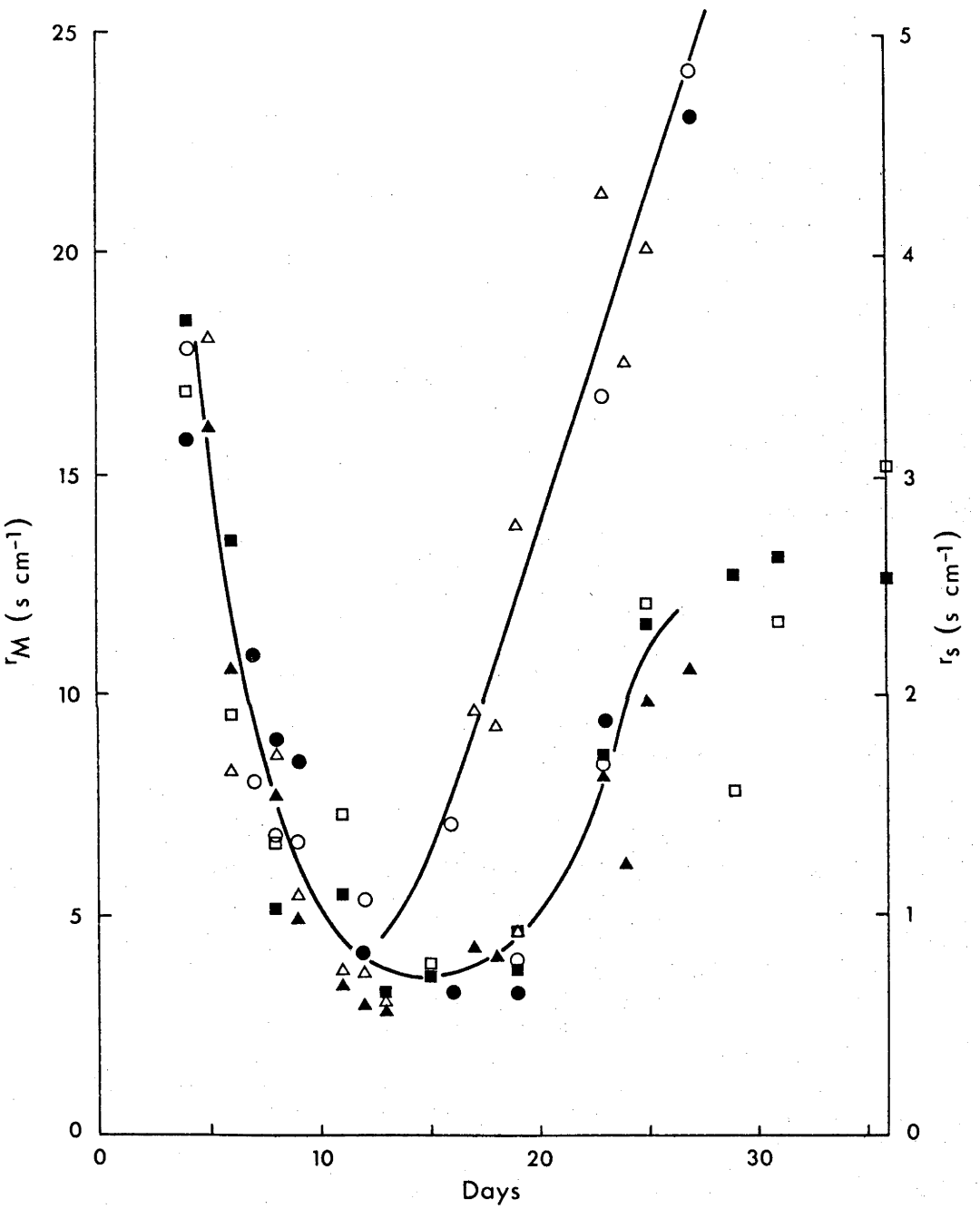

Fig. AP3. Stomatal (open symbols), and mesophyll diffusive resistances (closed symbols) for tobacco leaves expanding under high light (● , ○), low light (■ , □) and intermediate light with high nutrition (▲ , △)

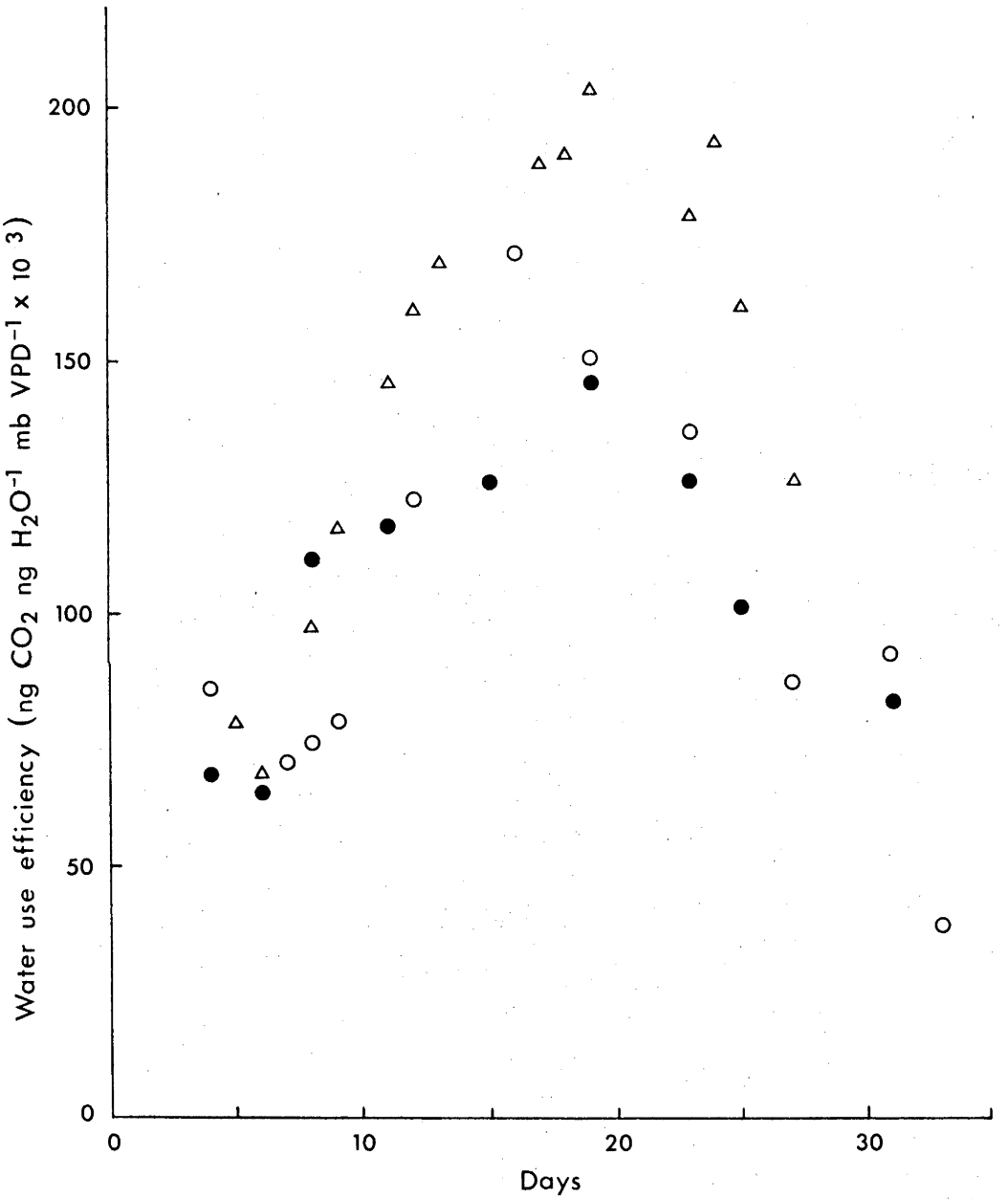

Fig. AP4. Efficiency of water use by tobacco leaves expanding under high light (○), low light (●) and intermediate light with high nutrition (△)

in all treatments indicating again that r_M changed more rapidly than r_s (in this case they increased). Peak water use efficiency was achieved when r_s rose and r_M remained relatively stable between days 15 and 20.

Water use efficiency was greatest under high nutrition, a fact well documented from fertilizer trials (e.g. Ballard 1933; Viets 1962) which could mean that ω can be used to complement this type of trial. At this stage however, it is not clear how ω is affected when measurements are made under changing VPD, but the linearity of transpiration between 40-80% relative humidity (Nevins and Loomis 1970, with sugar beet and Whiteman and Koller 1967, with *Atriplex*) indicates that comparisons can reasonably be made within this range. Measurements of sunflower leaves almost at A_{max} gave values for ω of around 0.2 ng CO_2 ng H_2O^{-1} mb VPD^{-1}, which is similar to tobacco. Changes in the water use efficiency of soybean leaves are presented in Section C.

Photosynthesis and Stomatal Resistances to Water Vapour Diffusion in Sunflower

For the distal portions of both surfaces of sunflower leaves, F rose rapidly to a maximum very early in leaf expansion and thereafter remained almost constant (Fig. AP5). There was no decline in F as found in tobacco. Resistance to water vapour diffusion similarly fell rapidly during the early stages of leaf expansion and remained low at least till A_{max}.

DISCUSSION

The first aim was to find if the timing of F_{max} in relation to A_{max} could be altered by growing conditions in a single tobacco variety. F_{max} was reached when the leaves were between 65 and 80% fully expanded.

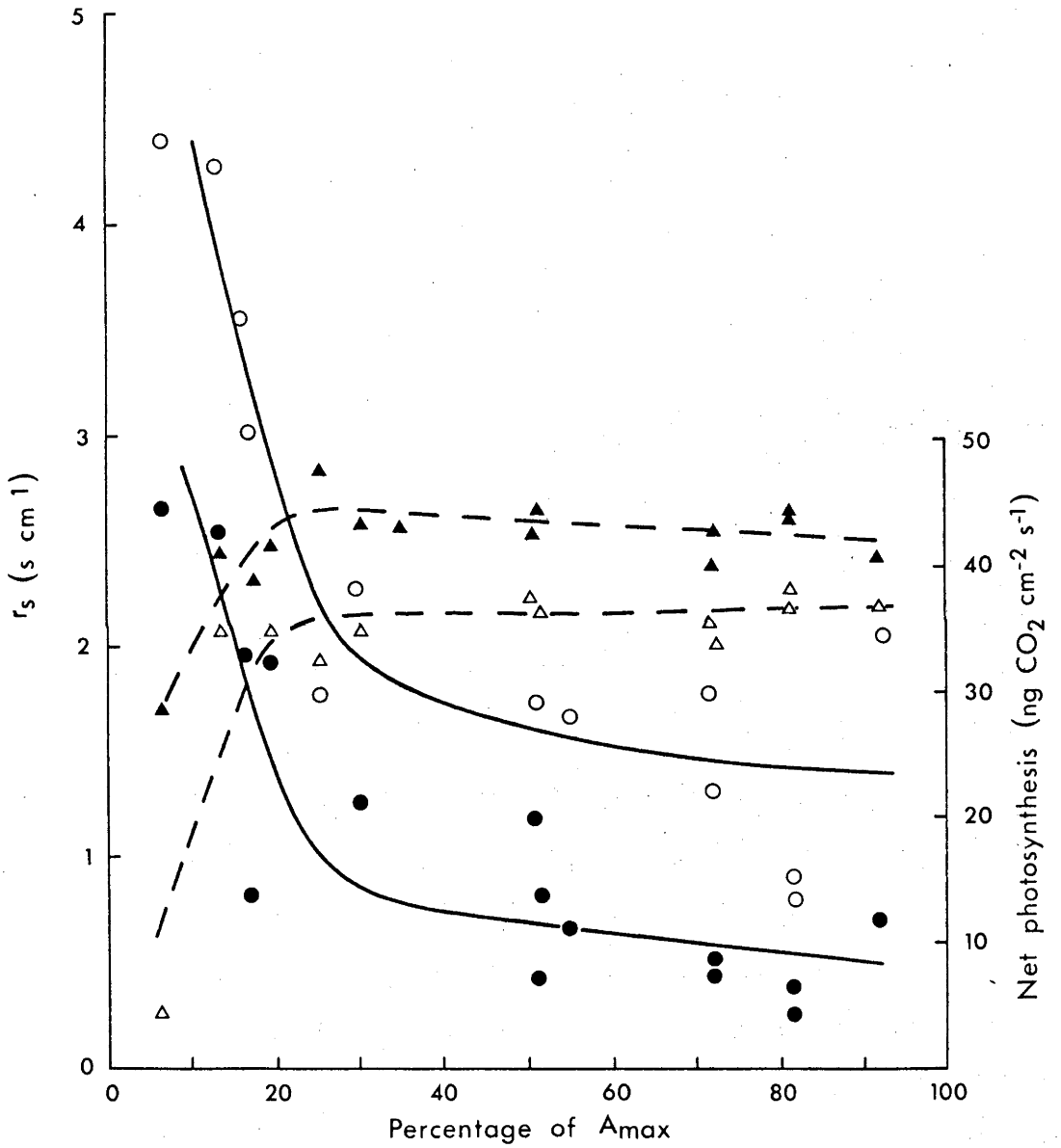

Fig. AP5. Net photosynthesis (△) and stomatal resistances (○) to H_2O vapour diffusion during leaf expansion for the adaxial (open symbols) and the abaxial surfaces (closed symbols) of sunflower leaves

This range, induced by different conditions of irradiance and nutrition, was a result of changes in the expansion characteristics of the leaves and not of changes in the photosynthesis pattern with time. In all treatments, F_{max} was reached a constant number of days after leaf expansion commenced, and further, F_{max} occurred on the same day as in the earlier studies in this laboratory when conditions of irradiance and nutrition were different again (Rawson and Hackett 1974). The one constant feature in all these studies was temperature, so it is tentatively concluded that temperature has an important influence in the timing of F_{max}. In contrast, Wada's (1968) four tobacco varieties reached F_{max} at 16 to 20 days (compared with 13 days in this material; his day 0 is equivalent to day 8), but his growth temperatures were lower and development would have been slower. He found that F_{max} was reached when the leaves were between 66 and 84% fully expanded.

The second aim was to determine which resistance(s) was associated with changes in F prior to A_{max} in tobacco (cf. also Wada 1968). There appeared to be three distinct periods in the relationship between r_s and r_M during expansion of the leaves. In the first, when F increased to a peak, and in the third, when F declined from 50% of F_{max}, relative changes in the two resistances were similar. During these periods r_M was from 3 to 5 times greater than r_s. In the second period when F started to decline, increases in r_s and r_M were similar in magnitude (around 2 s cm^{-1} in seven days) but the relative increase in r_s was greater.

Tobacco is apparently an unusual dicotyledon in having this important r_s component. Hodgkinson (1974) found that r_s scarcely changed when lucerne leaves aged or when they were rejuvenated by the removal of younger leaves and cf. Woolhouse (1968) and Ludlow and Wilson (1971b) for the magnitude of r_M in other dicots. Therefore it is interesting to

speculate as to what plant factors influence r_s in an expanding tobacco leaf and thus why F is reduced after F_{max} (cf. Hodgkinson 1974 for a discussion on the internal control of r_M by the plant).

Abscisic acid (ABA) and phaseic acid (PA) can influence stomatal aperture and the quantity of these substances in a leaf has been linked with the presence of a sink (Jones and Mansfield 1970; Loveys and Kriedemann 1974). Loveys and Kriedemann found that when the sink (a grape cluster) was removed from a vine, r_s values in an adjacent leaf rose from 1.41 to 7.14 s cm^{-1} in seven days, and ABA and PA increased concomitantly. However in a similar experiment, they showed that absolute changes in r_M were greater than those in r_s and that r_M remained the dominant resistance after sink removal.

Is there evidence that r_s and r_M in the tobacco leaves increased after F_{max} because of a reduced sink for assimilates? The plant's main sink at the growth stage being considered here is the developing leaves (Hackett and Rawson 1974a). These require imported assimilates only to the stage where they can support themselves, and as tobacco leaves export at their fastest rate when they are at about 50% A_{max} (Shiroya *et al.* 1961), this period of import must be quite short. It can be calculated from the present data and the growth analysis of Hackett and Rawson (1974a) that leaf 12 at day 13 required less than 30% of its photosynthate to support its growth. As the leaves expanded for at least 25 days excess carbon would have been produced if F_{max} had been maintained and had not declined. Hackett (1973) calculated that the tobacco plants of Petrie *et al.* (1939) had spare photosynthetic capacity to grow to twice the size realised. However, the argument that F declined because of sink limitation is invalid unless F can be increased when the sink is increased. Shading all leaves but one on tobacco plants increased F of

the irradiated leaf (Hackett and Rawson 1974b) demonstrating that tobacco leaves do have the capacity for increased photosynthesis.

The maintenance of F_{max} in sunflower leaves can also be explained in terms of assimilate requirement. Although tobacco and sunflower have a similar habit their growth is quite different under similar conditions. Sunflower plants reach floral initiation much earlier (25 vs. 60 days), they have a faster rate of leaf emergence (2-3 per day vs. 1 every 2 days at 25 days), stem elongation starts earlier, and the head is a massive structure compared with the small inflorescence produced by tobacco. (These values are approximate and those for sunflower are from unpublished work of Rattigan and Hackett). Therefore sunflower has a large sink throughout its life whereas tobacco does not.

In Section C of this thesis photosynthesis data are presented which reinforce the hypothesis presented here. The data are for soybean, another dicotyledonous plant with a different growth habit and a large sink during the latter half of its growth.